An evaluation of a proposed approach for overcoming the environmental and economic challenges of anaerobic digestion process through the production of more bio-products

A Thesis Submitted in Fulfilment of the Requirements for the Degree of

Doctor of Philosophy (PhD)

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DECLARATION

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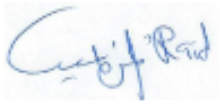

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Symbols	Description
AD	Anaerobic Digestion
ASTM	American Society for Testing and Materials
BBD	Box-Behnken Design
BVS	Bio-degradable Volatile Solids
CCD	Central Composite Design
CEN	European Committee for Standardization
CHP	Combined Heat and Power system
COVID-19	Corona Virus Disease
C/N	Carbon to Nitrogen Ratio
DCU	Dublin City University
DOE	Design of Experiment
DSC	Scanning Electron Microscope
EIA	Energy Information Administration's
EPA	Environmental Protection Agency of Ireland
FAO	Food and Agricultural Organization
FTIR	Fourier-Transform Infrared Spectroscopy
FVW	Fruit and Vegetable Wastes
IEA	International Energy Agency
IEO	International Energy Outlook
ILUC	Indirect Land Use Change
ISO	International Organization for Standardization
MSW	Municipal Solid Waste
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PA11	Polyamide 11 plastic
PE	Polyethylene
PHA	Polyhydroxyalkanoates plastic
PHB	Poly-3- Hydroxybutyrate plastic
PLA	Polylactic Acid plastic
PP	Polypropylene polymer
PS	Polystyrene polymer
PVA	Polyvinyl Alcohol polymer
RSM	Response Surface Methodology
RVS	Refractory Volatile Solids
R&D	Research and Development
SEAI	Sustainable Energy Authority of Ireland
SEM	Scanning Electron Microscope
TDN	Total Digestible Nutrient
TPS	Thermo-Plastic Starch
VFA	Volatile Fatty Acids
WWTP	Waste Water Treatment Plant

LIST OF NOMENCLATURE

Symbols	Description
Adeq. Precision	Adequate Precision
Adj. R^2	Adjusted R^2
ANOVA	An Analysis of Variance
B_P	Quantity of biogas produced for each gram of VS of the biomass [$m^3 g^{-1}$ VS].
B_S	Energy content of the biogas produced by biomass [kWh m^{-3}].
Cor. Total	Total sum of the squares corrected for the mean
Df	Degree of freedom
E_C	Electric energy consumed during the digestion to process 1 g of VS of biomass [kWh g^{-1}VS].
Energy gain / loss	The difference in percentage between the energy provided by the biogas produced from biomass (E_P) and the total electric energy consumed in the process. If the (E_C) is > than the (E_P), that's means the AD of the biomass is not economically feasible.
E_P	Energy of the biogas produced from 1 g of VS of the biomass [kWh g^{-1}VS].
E_{PT}	Electric energy consumed during the digestion measured by a kilowatt hour meter [kWh].
F value	A statistical test compares between two variances (model variance with residual variance)
g-VS	Gram volatile solids
HRT	Hydraulic retention time
ISR	Inoculum to substrate ratio
Ms	Moisture content
Net E_P	Total energy produced by 1 g of VS of biomass pre-treated.
Pred. R^2	Predicated R^2
T_g	Glass transition temperature
T_m	Melting temperature
Ts	Total solid
VS	Volatile solid
VS_m	Total quantity of VS into the flask [g].

LISTOF FIGURES

LIST OF TABLES

AUTHOR'S PUBLICATIONS

- **Published Journal Paper**

Alrefai, R., Benyounis, K.Y., Stokes, J., Integration Approach of Anaerobic Digestion and Fermentation Process Towards Producing Biogas and Bio-ethanol with Zero Waste: Technical, J. Fundamental Renewable Energy, 2017. 7(6): p. 1-6.

Alrefai, R., Benyounis, K.Y., Stokes, J., An Evaluation of the Effects of the Potato Starch on the Biogas Produced from the Anaerobic Digestion of Potato Wastes. J. Energies, 2020, 13(9), 2399; p. 1-24.

- **Published Book chapter**

Alrefai, R., Alrefai, A. M., Benyounis, K. Y. and Stokes, J. (2020). The Production of Biogas, Biodiesel as High-Value Bio-Based Product and Multiple Bio-Products Through an Integration Approach of the Anaerobic Digestion and Fermentation Processes. In: Hashmi, Saleem and Choudhury, Imtiaz Ahmed (eds.). Encyclopedia of Renewable and Sustainable Materials, Vol. 1, Oxford: Elsevier, pp. 686–694.

Alrefai, R., Alrefai, A. M., Benyounis, K. Y. and Stokes, J. (2020). A review on the production of thermo-plastic starch from the wastes of starchy fruits and vegetables. Encyclopedia of Materials: Plastics and Polymers. S. Hashmi. Oxford, Elsevier: 1-16.

Alrefai, R., Alrefai, A. M., Benyounis, K. Y. and Stokes, J. (2020). A comparatively study of the properties of the bio-plastic sheets produced from three conventional and unconventional starch sources. Encyclopedia of Materials: Plastics and Polymers. S. Hashmi. Oxford, Elsevier: 1-20.

- **Conference Paper**

Alrefai, R., Alrefai, A.M., Benyounis, K.Y. and Stokes, J., (2018), "An integration approach of anaerobic digestion and fermentation process to produce high-value bio-based products and multiple bio products", Proceedings of the 11th International Conference on Sustainable Energy & Environmental Protection (SEEP 2018), UWS, Paisley, UK, 08-11 May 2018, Vol. 2, pp. 7-12

- **Journal Paper under review**

Alrefai, R., Alrefai, A.M., Benyounis, K.Y. and Stokes, J. "Enhancement of the economic viability of anaerobic digestion through exploiting the whole biomass of mango waste and its residuals after digestion". Under review in Energies. Special issue of: Feature Papers in Sustainable Energy. Impact Factor: 2.702.

An Evaluation Of A Proposed Approach For Overcoming The Environmental And Economic Challenges Of Anaerobic Digestion Process Through The Production Of More Bio-Products

ABSTRACT

The transformation to renewable energy has become a requirement nowadays. Thermo-plastic starch (TPS) is a type of bio-plastic. Its quality and properties are examined by employing mechanical, physical, and other tests. Despite the proven advantages of using anaerobic digestion (AD) for the conversion of biomass into biogas and the developments on it, there still exist some environmental and economical challenges limiting prosperity and sustainability of AD. Economically, compared to fossil fuel market, biogas, which is the only major product of AD, is not competitive enough compared to the prices of its counterparts. With the aid of Design Expert software, the present study mainly aims to design and implement an integrated approach so as to potentially overcome these challenges and to make the AD more desirable. The approach incorporates the AD process with the process of producing TPS to form; biogas, bio-slurry and TPS/mango fibre bio-plastic sheet, from the residues of three starchy biomass: potato, mango and avocado. A Hollander Beater was employed as a multi-functional device, to: pre-treat the biomass, isolate the starch and to process mango seed coats. The study found that, the highest energy gain per gram of volatile solids of potato residues was 62.9% at 35 ^{0}C, 1.62 g-VS organic concentration and 50% sludge concentration, which yielded a maximum CH_4% of 72.4%. While, the highest energy gain by the gram of volatile solids of mango and avocado residues were 65% and 16.5% which yielded a maximum CH_4% of 62.4% and 60.9% respectively. The production of a bio-plastic sheet with a dimension of 2440*1220*3.2 mm has the same specifications of the optimal bio-plastic sheet produced, resulting in a cost efficiency of up to 65%. To achieve this approximately 353.7 kg potato, 89.9 kg mango and 173.7 kg avocado requires processing. Therefore the proposed study has achieved its main aim. Economically, this was achieved as a result of an excess amount of the bio-energy been produced (approximately 29%) of the total bio-energy produced. Regarding the environmental challenges which mainly depend on the accumulation of large amounts of the generated digestate, the study has confirmed the bio-fertiliser potential of the digestate with a suggestion to carry out more tests to confirm its quality and increase the reliability. Countries such as Saudi Arabia which is emerging in this field, can greatly benefit from this study via identifying the obstacles, tackling and avoiding them to improve profitability.

Chapter 1 : INTRODUCTION

1.1 Introduction

Due to the increase in the global population and others, the global energy demand has also increased. According to the Energy Information Administration's (EIA) in 2019, the global supply of crude oil, other liquid hydrocarbons, and bio-fuels is projected to be sufficient to meet the global demand for liquid fuels through to 2050 [1]. While, 85% of the world energy are derived from fossil-based fuel, they also have major threats not only on the environment but on human, animals and living organisms. Fossil fuel sources are not limited to only producing energy. They are also extensively used in manufacturing of many industrial materials, chemicals and polymers. The excessive use of fossil fuel sources, would lead to an earlier depletion of its sources and increase its negative contribution on the global environment. Renewable products and energies have the potential to offer promising solutions to fossil fuels, as they are derived from renewable matter and have a much less negative impact compared to fossil fuels. Recently, the dependence on the renewable sources have remarkable increased. The interests towards renewable products and energies are gaining much attention. For instance, over the period of 2010-2014, the global production of renewable energy increased by more than 40% and reached 128 GW [2, 3].

Furthermore, conventional plastics are polymers derived from petroleum resources. It is currently utilised in almost all aspects of life. These plastics represent a major pollution source and cause serious environmental problems, taking hundreds or often thousand years to degrade [4, 5]. According to Pathak, S. et al. [6], humans generate approximately 34 million tons of plastic waste per annum. Only 7% of the wastes are recycled and the remaining waste are disposed of in landfill, oceans, etc. Consequently, plastic pollution affects are not limited only to the environment, but also affects humans, animals, fish, marine animals, living organisms, and others. In addition to all of the negative impacts of fossil fuel, the long life of conventional plastics, before they totally decomposed as well as its high emissions of CO_2, are some of the main factors which have made conventional plastic less favoured today [7]. Figure 1 outlines the anticipated size of the mismanaged plastic waste by region in 2025. As is clear, it is anticipated that, the size of waste in the East Asia and Pacific in 2025 will be the highest compared to other regions, while the volume of waste in the North America anticipated to be the

least [8]. Lebreton, L. C. et al. [9], estimated the input of plastic to the oceans from the most polluting rivers across the world. Figure 2 shows the top 20 polluting rivers which accounted for approximately 67 % of the world and the annual river input of plastic into oceans. From this figure, it can be observed that the majority of the most polluting rivers are located in Asia.

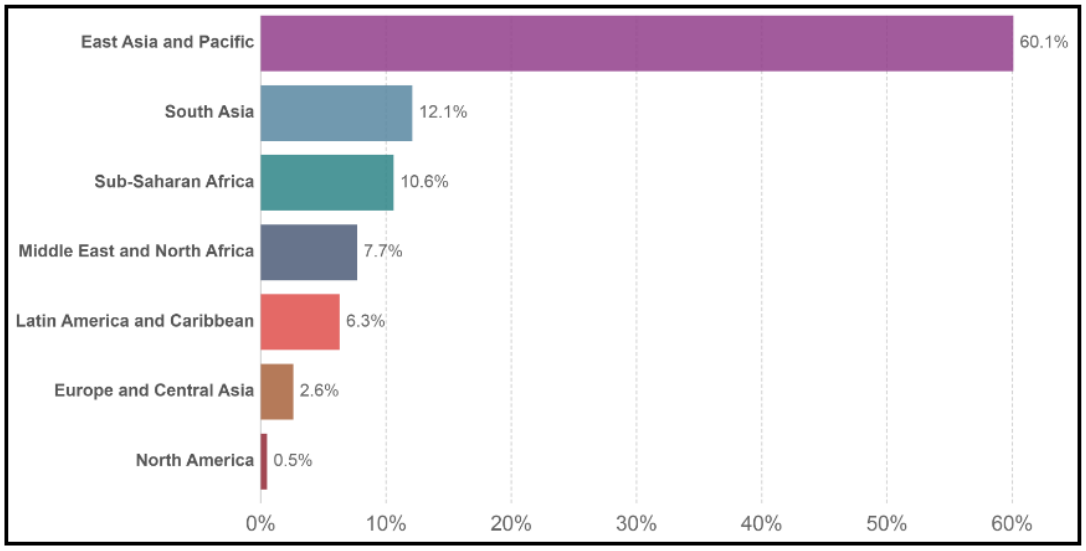


Figure 1: The anticipated size of the global mismanaged waste in 2025 by region [8].

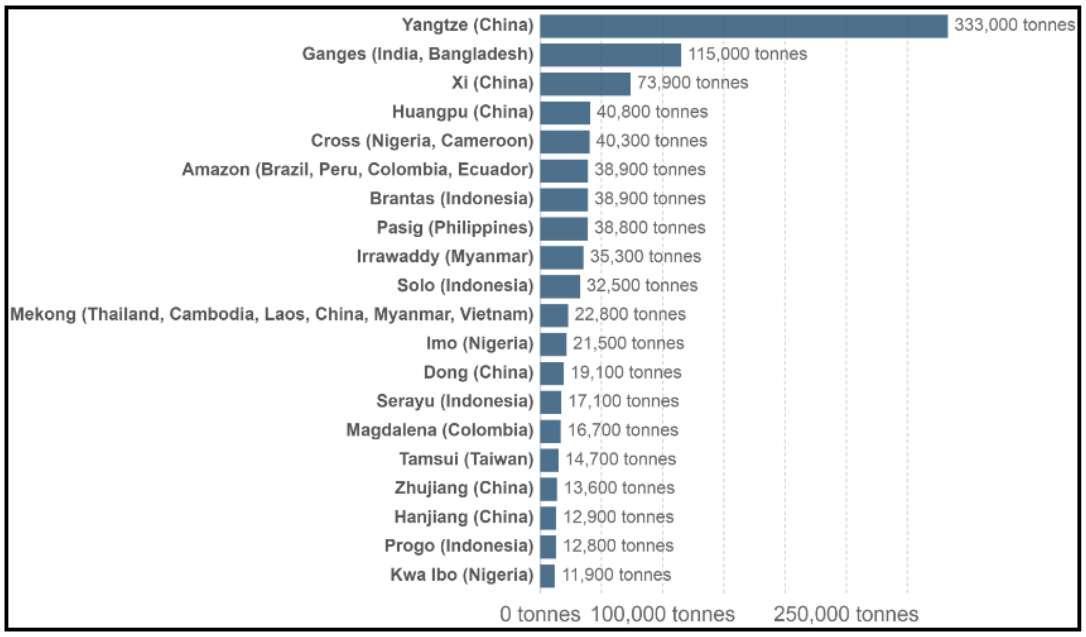


Figure 2: The annual river input of plastic into oceans [9].

Biopolymer is one of the bio-products derived from organic feedstocks. They are currently used in several applications like; textiles, packaging, electronics and food services. For example, about 66% of the bio-plastic produced in the world are

used in packaging applications [10]. There are several common types of bio-plastics that are commercially used. Starch-based bio-plastic or as it is well known "Thermoplastic starch" (TPS) is one of these types. It is commonly used in packaging applications (i.e. food packaging). Starch is the main raw material of this type of bio-plastic. It is tasteless, odourless, soft and comes typically in granulated powder form. It is produced by many plant species during photosynthesis. Normally, starch is located in the roots, pulps, seeds and tubers of certain plants in varied concentrations [11-13]. Fruits and vegetables are some of the most important sources containing starch in varying amounts. The production of TPS does not require sophisticated equipment nor tools and does not take long to process. Various methods and tests are usually applied to measure the quality and properties of the bio-plastic. Moreover, the poor properties and the brittleness of TPS have impeded the wide application of starch in real applications. The addition of polymers, bio-fillers or bio-fibres to the starch are some of the ways used to enhance the properties (i.e. mechanical, thermal, etc.) of the TPS, therefore it overcomes the poor process-ability and product performance of the starch and/or adds functional properties [14, 15].

The abundant availability of the natural fillers and fibres in almost every plant, their flexibility during processing, their biodegradability, the low relative density, the good strength and stiffness, and their low costs, are the reasons for the growing interest in using natural fillers and fibres as reinforced materials with bio-plastic to enhance its properties. The influences of the bio-fibres on the TPS properties and quality, are varied depending on the fibre type, environmental conditions (where the plant fibres are originally sourced), processing methods, and modification made to the natural fibre [16]. Bledzki, AK. and Gassan, J. [17] and Faruk, O. et al. [16], reviewed the reinforcement of the most readily used natural fibres in polymer composites pre 2010. Further studies are required to discover and examine the potentials of more bio-fibres and to overcome some obstacles such as; moisture absorption, inadequate toughness, and reduced long-term stability for outdoor applications. Furthermore, there are different processing technologies commonly used in producing TPS/ natural fibre composite such as; compression moulding, injection moulding, and extrusion. Compression moulding technology is applicable in the manufacturing of both thermoplastics and thermo-set plastics. Therefore this

technology, uses raw materials in a powder form, placed in the mould cavity, which undergo a certain pressure and heat, applied to the mould for specific time [16].

Biomass is the oldest and fourth largest source of energy after coal, petroleum, and natural gas [18]. It is still the largest source of renewable energy, specifically in developing countries [3]. Biomass is a sustainable and clean source of energy that has multiple advantages over non-renewable energy sources [19]. Lignocellulose biomass is an abundant source of biomass. It is classified into various groups such as, MSW, agricultural residues [20]. It is primarily waste materials containing carbohydrates (i.e. food residues) and non-competitive with food chains [21]. Biomass such as, Lignocellulose materials and others are widely used as raw substrates in several applications to produce diverse bio-products such as, bio-fuel, and biopolymer [22].

Anaerobic digestion (AD) is one of the most common effective bioconversion process to convert biomass into biogas. It has been widely utilised to convert large variety groups of biomass such as: food and agricultural residues and algae [23, 24]. Studies are still undergoing to explore and investigate the potentials of more biomass through AD process. Despite the significant effectiveness of AD among all other conversion process, AD could have major environmental issues, due to the high amounts of residues generated if it was applied extensively [25, 26]. From an economical point of view, numerous studies have reported that, the production of biogas alone from AD can be not economical competitive due to the volatility of fossil fuel price indices [3, 27]. Additionally, economical feasibility is the biggest challenge of the AD in terms of capital investment, operating costs, and revenues from the two bio-products of AD (biogas and bio slurry) [28, 29].

The pre-treatment process is a very crucial stage in AD. It aims essentially to enhance the bio-digestibility of the biomass in order to increase the total methane yield [30]. Pre-treatment methods can be classified as physical (mechanical), physico-chemical, chemical, and biological methods [31]. Beating pre-treatment as a mechanical pre-treatment was first introduced by the bio-energy group team in the School of Mechanical and Manufacturing Engineering in Dublin City University (DCU) based on employing a Hollander Beater [32]. It has recently proved its

significant potential in pre-treating different types of biomass such as; seaweeds, maize silage, fresh grass and potato waste [32, 33].

Biogas and residues are the two main products produced from AD. AD residues contain of microbial biomass and undigested material. Any leftover material does not decompose nor converts to biogas, remains post to the AD process in the residues as solid and liquid streams. AD residues are commonly known as "digestate". Digestate consist of valuable nutrients of different amounts based on the feedstock processed. It can be either used directly or it can be processed and then used in multiple ways such as: a source of animal feeding, organic fertiliser, etc. [3, 34]. Despite the proven bio-fertilisers potential of the digestate in many cases, it can contain harmful elements. Thus, the contents of the digestate compared to the harmful elements must be measured prior to using it in agricultural applications or other applications [35]. Additionally, there are some other factors limit the uses of digestate such as; the high costs for storing it, transporting it and applying it [36].

AD biogas is a promising renewable alternative for natural gas. It can be generated from many different species of organic wastes [37]. Its composition is quite similar to landfill gas but different from natural gas. It consists of a varied quantity of gases. The compositions of biogas are different from one to another based on the feedstock, the organic matter load and the feeding rate of the bioreactor. Methane gas typically represents the largest amount of gas contained in biogas followed by CO_2 [38, 39]. Biogas could contain some traces of impurities such as; hydrogen sulphide H_2S. Removal of these impurities can be done by employing an appropriate gas upgrading technology [40]. Compared to the calorific value of a natural gas, the calorific value of the typical AD biogas ranges from 5.5 to 6.5 kWh/m^3, while natural gas has an energy value of 5.8-7.8 kWh/m^3 [41]. Moreover, biogas can be used in all natural gas appliances. It can be also applied in numerous applications like, combined heat and power system (CHP) [39]. In Europe specifically, biogas is normally used for producing heat and electricity [37].

A Design of Experiments (DOE) is a statistical approach that helps to identify the significant parameters and their interactions which influence a process. It greatly helps in obtaining any improvements to the process, as well as identifying the optimal settings for the process in order to attain top performance. DOE provides

useful information regarding the process being studied, using multiple experimental runs. Among all of its useful features, it simplifies the analysis process as it provides a clear image on the process and the results. Therefore, there are now many studies are employing this technique.

As mentioned previously, AD could contribute to the major environmental issues due to its huge residues, which can be accumulated if it was applied at large scale. A recent study of the production of biogas from Irish seaweed [33], suggested that, in order to obtain more comprehensive understanding of the economics of the process, the energy consumption related to the whole AD process including (digestion and pre-treatment processes) should be investigated. This would help also in evaluating the economic feasibility of using biomass to produce biogas on a large-scale. Fossil fuel markets and prices indices are usually fluctuating. The production of biogas through AD requires relatively high energy, particularly due to the pre-treatment and digestion steps. Due to all of that, when AD is used to produce only biogas and bio-slurry, AD becomes economically unattractive. Thus, alternatives are required to overcome such obstacles, in order to make AD economically feasible [3, 27]. Furthermore, the bio-refinery concept is similar to conventional petrochemical refinery in which multiple products are produced from crude petroleum. Therefore, an integration approach can correspond to that used in a bio-refinery concept [3, 42]. However, an integrated approach is one of the solutions for those issues. Many integrated approaches of several combinations of bioconversion technologies and bio-product production processes have been investigated and studied using different feedstocks (i.e. AD with fermentation process for producing biogas and bio-ethanol). However, more studies are crucially needed using this approach for it to be prosperous [43]. An integrated approach involves incorporating bioconversion technologies together or with bio-product production processes into one approach to produce multiple bio-fuels and bio-products such as: biogas, bio-plastic, bio-ethanol, biodiesel, etc. Essentially, it aims to optimise the profit by producing low volume high-value products, meeting the energy needs by producing low-value high volume fuels and minimising the waste streams generated. In addition to that, some types of this approach aim also to make full use of biomass, by using the whole biomass including all its by-products to

produce multiple bio-products [3] such as; using the potato peels and the separation of starch from them to produce biogas and bio-plastic.

Scientific literature contains several studies on the integration approach of multiple bioconversion technologies, bio-product production processes. However, the author was unable to identify any study focused on an integration approach of AD and TPS. Most studies in literature focused on the production of TPS, utilising the starch isolated from the most common primary starch sources (i.e. corn, cassava, potatoes and bananas) as raw materials. The scientific literature review of the characteristics of the starch extracted from the non-conventional sources such as; fruit seeds are quite low [44]. Additionally, only few studies have been found on the production of TPS from mango and avocado starch, hence the reason these substrates were identified and used in this study.

1.2 Significance Of The Research

The dependence and demand today on energy is higher than ever before and expected to continue to increase [18]. Compared to 2011, the International Energy Agency (IEA) has estimated a 70% and 60% increase by 2050 in both the global energy demand and greenhouse gas emissions respectively [45], while, most of the energy consumed worldwide are from non-renewable energy supplies [46]. The continuing use of non-renewable energy at this rate will increase its negative impact and deplete their resources more rapidly [2, 45]. Generally, most studies related to renewable energy aim somehow to reduce the dependence on fossil fuel [47], that is because the reduction in the use of non-renewable energy reduces its negative impacts on the environment, health and on the quality of life in particular. On the other hand, the use of renewable energy is receiving more attention [45, 48]. Due to the advantages of the use of renewable energy over the fossil fuel, plus as the awareness of the harmful impacts of fossil fuel has increased, the use of renewable energy is expected to increase in the future [48]. Biomass energy is a form of renewable energy, furthermore, it can be converted into solid, liquid and gaseous bio-fuels for generating bio-energy by one of the bio-conversion processes. Biomass can also be converted into some bio-chemicals and biomaterials. However, comparing biomass to solid fossil fuel, it has low energy densities and is less efficient [49], but further investigation will make it more viable

Anaerobic digestion (AD) is a multi-function bioconversion process of diverse organic substrates into biogas. Beside its main objective to produce bio-energy, it plays a crucial role in the stabilising and management of wastes [3, 27]. Generally, fruits and vegetables are some of the highly consumed foods. While the consumption rates vary from one to the another, the volume of both fruits and vegetables residues which are normally disposed of, is quite large [50]. For example, the edible parts of a mango fruit can constitute approximately from 33 to 85% of the total fruit. As the rest is usually disposed of, they are considered waste [51]. Fruit and vegetable residues typically contain reusable by-products, but these by-products are not extensively commercially exploited [50]. Starch is a natural biopolymer considered as a by-product of many fruit and vegetable residues. Its physicochemical properties differ from one source to another. Corn, potato, rice, wheat and cassava, are considered as conventional sources of starch [52]. However, more studies are greatly needed to explore the properties of the starch of more sources like the fruit seeds and investigate their applications in industry [44]. In other words, the excessive use of the well-known starch sources has led to increased demand for exploration of new starch. Because of this, attention has grown towards starch over the last few years. Furthermore, the use of starch may be limited by some of the properties of the native form. Therefore, studies are under way to explore more starch sources and modify their properties for improving its functionality and make it a competitor to their counterparts [53].

AD is facing a number of challenges which have made the investment in it less attractive [29]. Compared to fossil fuel, AD typically requires larger amounts of feedstock to produce the same amount of energy produced by significantly less amounts of fossil fuel [49]. For instance, in the Republic of Ireland the availability of low cost waste feedstock can produce up to 126 thousand tonnes of oil equivalent (5.3 PJ) biogas/year, which is equivalent to approximately 3% of the natural gas supplied in 2015 in the Republic of Ireland. The fluctuation of fuel price indices, the operational cost of AD and the expenses on the digestate generated are some of challenges that adversely affect the economic viability of AD [3, 27, 29].

On the other hand, the use of large amounts of feedstocks to produce low volume of biogas compared to the energy volume produced from the same amount

of fossil fuel, result in a production of large amounts of digestate. The generation of digestate in large quantity lead to major threats on the environment [3, 25, 49]. In addition, there are several operational factors that also pose a challenge to the stability of the AD process. These factors can be avoided if they have been taken into consideration at an early stage of the AD [54, 55].

Furthermore, the determination of the influencing factors and their extents of influence on the quantity and quality of the biogas such as; the temperature and the concentrations of each materials being fed into the digesters, could significantly contribute in improving the quantity and the quality of the biogas and therefore increase its efficiency [55]. As previously mentioned, biogas as a single product of the AD is not economically competitive with its counterparts [27]. The production of more bio-fuels and bio-products at low production costs (i.e. bio-plastic, bio-ethanol, biodiesel, etc.) by taking advantage of the entire biomass could possibly increase the economic profitability of the AD [3, 42]. The composition of the digestate varies based on the feedstock used. The containment of the digestate to the basic nutrients of conventional fertiliser (N, P and K) enhances its use in agriculture [3, 56]. Thus, the exploitation of the digestate in agriculture or other applications greatly helps in limiting or perhaps eliminating the environmental challenges associated with AD [3, 25].

Based on the above, overcoming the environmental and economical problems associated with AD, and improving the quantity and quality of biogas and thus its effectiveness, will certainly make the investment in AD more desirable. Such a desirability will be accompanied by a reduction in dependence on fossil fuels, which will also lead to a reduction in the negative effects attributed to its use [3, 47].

In conclusion, the present study looks into overcoming these issues by increasing the economic profitability of AD process, and contributing in the reduction of the food waste generated by food processing industries, which represents the second largest generator of waste in the environment after household sewage [50]. Consequently, overcoming these issues will enhance the investment in AD, and therefore reduce the dependence on natural gas and eventually can efficiently substitute it with the biogas. Additionally, emerging countries in this field, such as the Republic of Ireland or a country like Saudi Arabia aiming to start

in this field, may benefit greatly from knowing of these issues, obstacles, and methods proposed to solve them.

1.3 Research Objective

This study essentially aims to design and implement an integrated approach analogous to bio-refinery concept. The three starchy fruit and vegetables which have been investigated in the present study are, potato, mango and avocado. The three fruit and vegetable biomass were selected according to a number of factors such as: their availability, their starch content and the possibility of combining them into one approach. The present study aimed to achieve the following major objectives:

- To achieve the scientific hypothesis of the study. Experimentally, this is achieved by measuring the quantity (biogas volume) and quality (the composition of the resulted biogas from the CH_4 and CO_2) of the biogas produced from each biomass in order to find the optimal biogas with an aid of the DOE to find the highest energy gain can be obtained from each biomass. Following that, the study is measured the properties of the TPS sheets produced and calculated the cost efficiency based on the results of the optimisation process which is carried out using DOE. Then, the study is theoretically incorporated the results of the energy balance and cost effectiveness to investigate the influence of the incorporation on the economic aspects of the AD.
- To incorporate TPS production process and AD to produce multiple bio-products: biogas as bio-fuel and, TPS/mango fibre bio-plastic sheet and bio-slurry as biomaterials.
- To minimise the waste generated through confirming the content of the digestate to the basic nutrients that conventional fertilisers have.
- To make full use of biomass.
- To realise one of the bio-refinery concepts that aims to integrate equipment to produce bio-materials, bio-energy, etc. through the use of the Hollander Beater as a multi-functional device, for: mechanically pre-treatment the biomass, isolate the starch and prepare mango seed coats as reinforced materials.

- To explore more starch and reinforced materials sources which have the potential to be used in producing bio-products to reduce the pressure on the current available known sources.
- To blend mango seed coat fibre as reinforced material with the three starch at varied concentrations to find out the optimal composite of starch and fibre at the optimal set through testing and comparing the quality and properties of each TPS composites produced.
- To find out the optimal set of parameters (temperature, sludge concentration and organic concentration) at the optimal biogas quantity and quality.
- To compare between the biogas quantity and quality before and after the separation of starch and mango coats to assess their impacts on the biogas produced.
- To calculate the energy gain and cost effectiveness to evaluate the economic feasibility of the integrated approach.

The experimental work was optimised by the DOE technique. DOE helped in specifying the influence of each factors on the process. The optimisation process and analysis were carried out using one of the DOE techniques namely Response Surface Methodology (RSM).

1.4 Thesis Outline

This report consists of six chapters. The outlines of each chapter are as follow:

Chapter 1: Introduction: it provides general introduction to the integrated approach of incorporating TPS production process and AD to produce multiple bio-products from the wastes of three different fruits and vegetables. The chapter also illustrates the aim of this study.

Chapter 2: Literature Review: this chapter provides the reader with the most important concepts regarding to the main topic of the study. It is segmented into eight major sections as the following: bio-plastic, characteristics of some starchy fruits and vegetables, renewable and non-renewable energy, AD, biomass pre-treatment, biogas, digestate and an overview of integration approach

Chapter 3: Experimental equipment and procedures are detailed in this chapter: it depicts the materials, equipment, and techniques which were used in the project. In

addition, this chapter gives a description and an explanation of the experimental procedures.

Chapter 4: Results and Discussion: this chapter presents the results obtained from the experiments and discusses them**.**

Chapter 5: Optimisation, energy balance and cost effectiveness: this chapter shows the optimal set of parameters at the optimal biogas and TPS and calculates the energy balance and cost effectiveness based on the optimal results.

Chapter 6: Conclusion and future work: this chapter presents the main conclusions and details the main suggestions for future works.

Chapter 2 : LITERATURE REVIEW

2.1 Introduction

Generally, bio-fuel or bio-energy is divided into three groups based on its forms, which are; 1) gaseous fuel i.e. biogas, 2) liquid fuels i.e. bio-diesel and bio-ethanol or 3) solid fuel for production of heat and power. Bio-products can be materials, chemicals and energy derived from biological or renewable materials. Biomaterials includes many bio-products in which are widely utilised in modern life such as; bio-plastic, adhesives, resins, food/feed ingredients, etc. [57, 58]

This chapter is divided into a number of sections, which reviews the scientific literature on the main topics relevant to the integrated approach of producing TPS composite, biogas and digestate from the residues of three starchy fruits and vegetables. In order to obtain the full knowledge of the prescribed integrated approach, the following topics are clearly described in this Chapter: (1) bio-plastics, starch-based bio-plastic and reinforcing materials; (2) the characteristics of some starchy fruits and vegetables; (3) the global demand of energy, renewable and non-renewable energy and some aspects of bio-fuel ; (4) AD process, AD feedstocks and lignocellulosic biomass; (5) different types of pre-treatment process of biomass; (6) biogas, and its compositions, uses and upgrading technologies; (7) digestate, and finally (8) an integration approach.

2.2 Bio-Plastic

Plastics are the most common polymers used nowadays. It is used in many applications (i.e. packaging) and made from petroleum materials [59]. Rivero, C.P. et al. [7], stated that the annual world production of conventional plastic has recently reached about 299 million tons. Where, only 5% of it is not petroleum-based plastic. According to Pathak, S. et al. [6], the estimation of global waste of plastic is around 34 million tons per year. About 93% of these wastes are disposed of in landfills and oceans as well. According to the bio-plastics market data report [60], in 2018 the bio-plastics in all their types represented approximately 1% of the annual production of plastic of 335 million tonnes. Furthermore, the report revealed that, the bio-plastic market is expected to continue growing from approximately 2.11 million tonnes in 2018 to around 2.62 million tonnes in 2023 due to the increasing of the demand to it.

Most commodity plastics used nowadays are either non-biodegradable or even take hundreds or thousands years to degrade [4, 5]. It is potentially toxic and can prove to be carcinogenic. That is due to the excessive chemical additives used in the production of plastic [61]. Petroleum plastic is one of the major causes of pollution. Plastic pollution can negatively affect lands, waterways and oceans. Human, animals and even living organism are also can be affected by plastic pollution. For instance, it can cause a disruption in the thyroid hormone levels for human [62]. However, due to all negative impacts of plastic and the threats of the depletion of fossil resources as well, the interests in the development of bio-plastic has greatly increased [7]. Bio-plastic is a type of plastics, derived from biomass sources, such as; corn starch, or microbiota. The biodegradability of bio-plastic in different environmental conditions and its uses of renewable sources i.e. agricultural wastes have made it more favourable than the conventional plastics. The biodegradability of it is significantly influenced by their physical and chemical structures. While, the environment in which the bio-plastic located, plays an important role in its biodegradation [63]. According to Anstey, et al. [64], many studies were implemented to evaluate the biodegradability of bio-plastics under different environmental circumstances, such as soil, compost, marine and other aquatic environments. Among these circumstances, soil and compost were most considered because of their high microbial diversity.

Bio-plastics are generally divided into biodegradable and bio-based. When the focus on the bio-plastic is at the end of its product life, is classified as biodegradable. While, when the focus is on the origin of the carbon building blocks, it is classified as bio-based. Actually, not all bio-based plastics are characterised as biodegradable and vice versa. Figure 3 differentiating between the biodegradable and bio-based plastics. It also shows the standard testing method used for determining the bio-based content of the materials and the specifications for the compostable plastics.

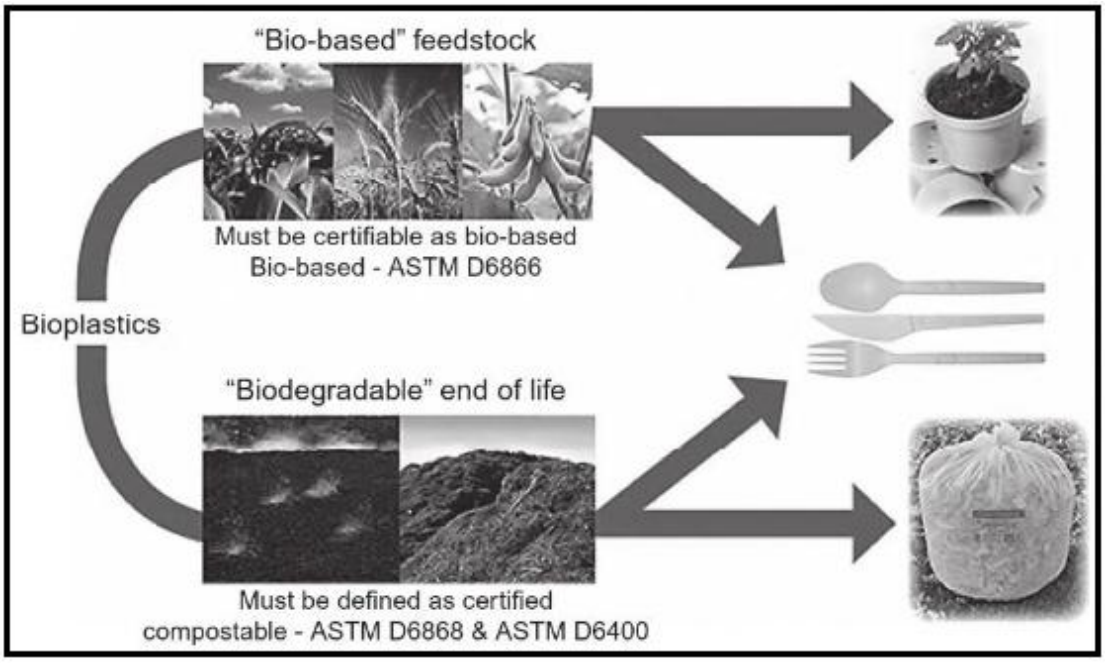


Figure 3: Differentiating between bio-based and biodegradable plastics [10].

In comparison to the bio-plastic, petroleum-based plastics are non-biodegradable plastics. The uses of this plastics mainly contribute in the environmental impacts due to the emissions of carbon dioxide and their long-period accumulation in the environment [6, 65, 66]. Bio-plastic has a number of advantages over the conventional plastic, some of them are; its emissions of carbon is very low compared to conventional plastic, it saves energy during production, does not use non-renewable sources, does not contain health-damaging additives, and does not change the flavour or smell of food when it is used for storing. Like any other materials, bio-plastic has some drawbacks which should be taken into account, such as; high cost of production and poor mechanical properties. The cost of production can be avoided by using low cost renewable resources like agricultural wastes [66, 67].

There are many types of bio-plastic and some of those that have been investigated widely, are; Aliphatic polyesters, polylactic acid (PLA) plastics, polyhydroxyalkanoates (PHA), poly-3- hydroxybutyrate (PHB), polyamide 11 (PA 11), starch-based plastic (thermo-plastic starch), cellulose-based plastics, bio-derived polyethylene and genetically modified bio-plastics [68, 69]. PHAs are non-toxic, biocompatible, biodegradable and have a high level of polymerisation and crystalline. PHAs have a great potential in the future due to their properties [70]. PHAs and PHBs and their derivatives are the most extensively produced microbial

bio-plastics [71-73]. The production of PHAs generally passes through number of stages, which are; fermentation, separation of biomass from the broth, drying of biomass, extraction of PHA, drying of biomass and finally packaging [74]. Among the natural polymers and after cellulose, starch is the second most natural polymers in term of abundance. Starch based plastic is very common type [75], which is described in more detail in the next section.

In general, the bio-plastic market has accounted for roughly about 1% of the global plastic market. However, it is projected to increase at a fast pace [76]. Furthermore, there are some factors that would significantly help in increasing bio-plastic market, which are; continuous research and development (R&D) activities, increased environmental awareness, and implementation of stringent environmental regulations. Biodegradable plastics are presently utilised in many different commercial applications such as; packaging, disposable house wares, bags and sacks, consumer electronics, and the automotive sector [76].

Moreover, Lignocellulosic biomass are widely available everywhere. It is one of the most promising biomass in production of bio-fuels and a number of bio-based products/chemicals such as, organic acids and bio-plastic [77]. Lignocellulosic biomass has a large potential for producing bio-plastic. It is originally composed of polymers in plant cell walls including Cellulose, Hemicellulose, Pectin, and Lignin along with low proportion of organic materials like proteins and lipids, and minerals [78]. Normally, Lignin provides rigidity to plant cell wall and resists to biological degradation. However, post the AD process of Lignocellulosic biomass, Lignin stays undigested and it can be then used in producing bio-energy such as, heat and electricity through thermo-chemical processes and different bio-based products such as, biopolymer and Vanillin through chemical processes [3, 27].

2.2.1 Starch-Based Plastic

Starch is a natural polymer. Where in industrial applications, starch is commonly used in the making of bio-plastics. It can be used alone or blended with other components such as: Agar as a reinforcement material for making hard bio-plastic. It is produced by many plant species during photosynthesis and starch stores energy for plants. Therefore, starch can be found in roots, pulps, seeds and tubers of plants in large concentration. It exists in many of our highly consumed

daily foods, i.e.; rice, potato and wheat. Starch is tasteless, soft and odourless carbohydrate comes commonly in the form of granular powder [11-13]. Starch could comes in several forms as well based on the origin of its raw materials [79]. Furthermore, Figure 4 gives an illustration on the life cycle of starch based bio-plastic which starts with the growing of the plants that are rich in starch following by harvesting the plants and extracting the starch, refining and fermenting the starch, etc.

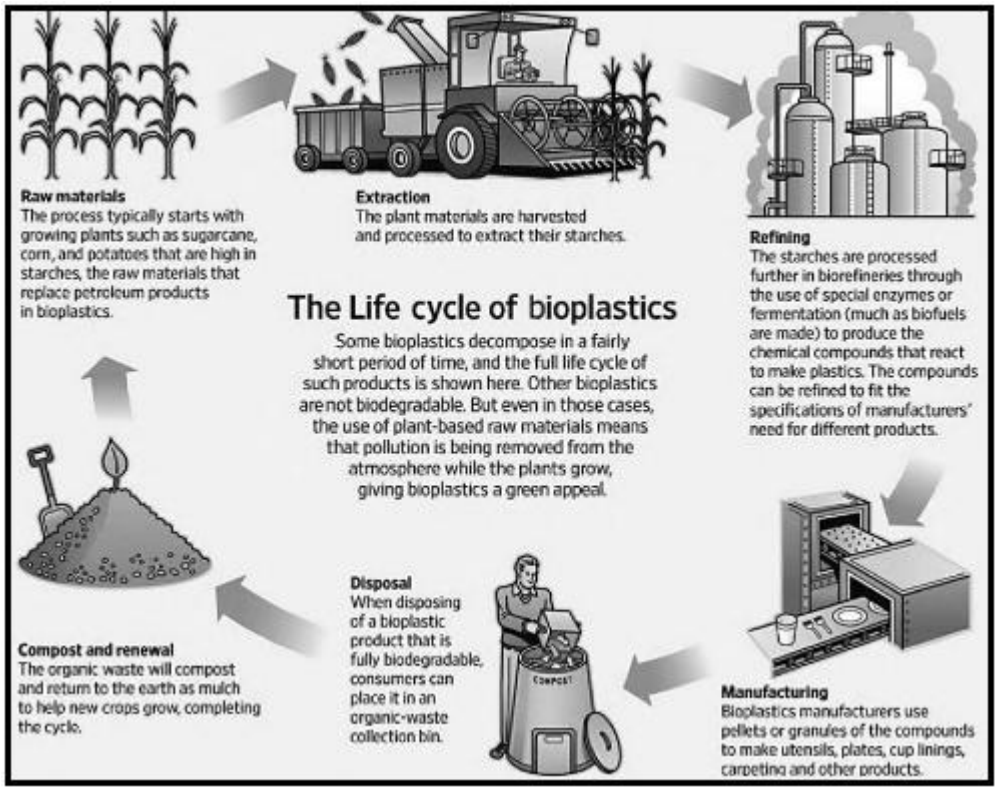


Figure 4: The life cycle of the starch based bio-plastic [10].

Starch mainly consist of two molecules; Amylose and Amylopectin. Amylose polymer is essentially linear chains molecules composed almost totally of α-1, 4 bonds, while, Amylopectin is a polymer with highly branch points joined by α-1, 6 bonds [80, 81]. These two chains have different physical and chemical properties [13]. They exist in varied proportions based on the plants. Most starch like potato starch, but not all, are composed of approximately 25% Amylose and 75% Amylopectin [82, 83]. Legume starch i.e.; pea starch contains high amount of Amylose. D-glucose or as commonly known Dextrose ($C_6 H_{12} O_6$) is the monomer unit in starch [84]. Amylose and Amylopectin structures can be decomposed by specific enzyme which degrade the polymer [85]. However, starch has proved its

significant efficiency in many bio-plastic applications. Due to that and as it is cheap, easy to extract, abundant, biodegradable and renewable, starch is one of the most important polymer used in bio-plastic production [86, 87]. On the other hand, there are some drawbacks of using starch as raw materials in bio-plastic applications. The main common drawback of using it is the high solubility of starch in water and the low mechanical strength. Overcoming this drawback and improving the properties of starch, involved many studies, such as blending starch with degradable synthetic polymers like; Polyvinyl Alcohol (PVA), Lignocellulosic fibres as reinforcement and Agro wastes [88-90]. Furthermore, Petrova, M. and Garner, J. [91], reached that, Poly-Lactic Acid (PLA) and starch-based plastics are the most influential commercial biodegradable and bio-based polymers. In 2012, they were represented for about 47% and 41%, of the total biodegradable polymer consumption respectively [92]. In 2018, the global production capacities of starch-blends type of bio-plastics were around 18.2% of the total global bio-plastics production capacity of all types of bio-plastics of around 2.11 million tonnes [60].

The majority of the bio-plastics produced in the world are used in packaging. They are expected to increase in the coming years. Starch based plastic is one of the most common bio-plastic types used in packaging applications [10]. It is made by native or slightly modified starch. These modified starch can be isolated or blended with other natural or synthetics molecules [87]. There are numbers of modifiers extensively used to plasticise starch including; glycerol, sorbitol, water, urea, ethanolamine and formamide. Water and glycerol are the most common plasterers used. They are very effective as they have the potential to be inserted easily into starch [93]. In the plasticising process of starch, the modifier breaks the internal hydrogen linkages between the glucose rings. At high temperatures of about (90 °C to 180 °C), the plasticiser converts starch to thermoplastic starch (TPS) and make it therefore able to be extruded, injected, moulded and pressed. As starch normally consists of linear Amylose and greatly branched Amylopectin, it is considered as crystalline material. When starch is blended with limited quantities of water and then subjected to heat and shear, it undergoes spontaneous destruction. A homogeneous melt known as thermoplastic starch (TPS), which has thermoplastic characteristics [12, 94]. The thermal properties of thermoplastic are different than thermo-set. When heating, thermoplastic is becoming soft and fluid and set when

cooling. In thermoplastics, the polymer chains are linear or branched and do not become cross-linked. While, thermo-set is obtained by cross-linking a linear polymer and will burn if it is reheated [85]. Furthermore, TPS material is eco-friendly, renewable, flexible and degradable and can be incorporated into soil as an organic fertiliser. It is made by the disruption of the starch granule structure when it is processed with low amount of water and with thermal and mechanical forces in the presence of plasticiser in which does not easily evaporate during processing [95]. The main role of plasticiser when incorporated into a plastic is to increases the flexibility and applicability of the plastic. So that, plasticiser molecules penetrate starch granules and therefore destroy the interior hydrogen linkages of starch in high temperature, pressure, and shearing [96].

The process of producing TPS is relatively simple. It does not require long processing times or even complicated chemical reactions. The quantity of plasticiser and water used in the process has a major impact on the properties and quality of bio-plastic. Thus, it should be carefully selected. The plasticiser has an impact as well on glass transition temperature (Tg) of TPS. Consequently, the type of plasticiser should be carefully selected too [12, 97]. In order to enhance the properties of TPS, it is incorporated with other polymers, fillers, and fibres [15].

TPS are gaining much attention nowadays because of its magnificent advantages including the renewability, the biodegradability and the cost reduction of TPS. Due to that, TPS can be found widely in many applications such as; food packaging (i.e. containers, wraps), disposable eating utensils, bags for trash, etc. Food packaging represents about two-thirds of total amount of plastic consumption. Actually, not all bio-based plastics are biodegradable. Some of them, act like petroleum plastic and take hundreds of years to be degraded. Practically, raw material from which a plastic was made does not determine if the plastic would biodegrade or is compostable. Where, the end of life characteristics do not depend on the quantity of bio-based content used to create a product. [98-100]

Furthermore, petroleum-based plastic and starch-based plastic are two types of plastics used in the production of plastic products. Keshav, S. [101], compared the advantages and disadvantages between both types as given in Table 1 and Table 2.

Figure 5 below also shows a comparison between the bio-plastic and the petroleum-based plastic.

Table 1: Advantages of Petroleum-based Plastics and Starch-based Plastic and the comparison between them [101].

Petroleum-based Plastic	Starch-based Plastic
• Lightweight - malleable - has the potential to be utilised in the production of some plastic products such as: carry bags.	• Lightweight - malleable - has the potential to be utilised in the production of some plastic products such as: carry bags.
• Has better physical properties and stronger mechanical properties	• It does not contribute in water and environmental pollution.
• Has better resistance to chemical compounds, therefore it can be used in the production of chemical compounds containers.	• It is degraded in short times (for example; potato starch-based bio-plastic takes approximately 90 days to be aerobically degraded in compost at 85% and 58 °C [102]).
• It can be applied as insulators in many appliances.	• Less energy required to be produced
• Able to be moulded into complex forms	• It is eco-friendly and does not cause harms to human, plants, marine, animals, etc
	• It helps in decreasing the dependency on petroleum plastic and can be the best substitute for it.
	• It derived from cheap, sustainable and renewable materials such as potato starch

Table 2: The disadvantages of Petroleum-based Plastic and Starch based Plastics and the comparison between them [101].

Petroleum-based Plastic	Starch-based plastic
• It derived from non renewable and sustainable materials such as crude oil.	• Its shelf life is relatively short.
• It generates huge solid wastes in which negatively contribute in increasing the pollutions	• It has weaker mechanical and chemical properties.
• It may take around hundreds or thousand years to be totally degraded.	• As comparison to petroleum plastic, its uses are limited
• It causes harms to human, plants, marine, animals, etc if swallowed. Many marine animals are killed every year because of the pollution caused by conventional plastic.	
• It emits harmful gases when it is burnt.	
• Some types like; polycarbonate (Bisphenol A, BPA), can cause health problems.	
• The production of it required intensive energy	

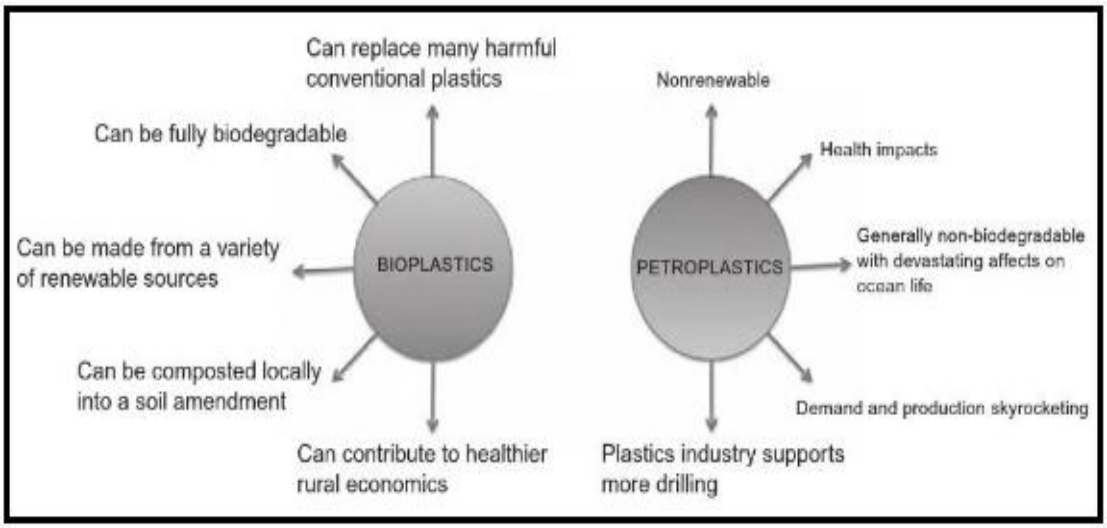


Figure 5: A comparison between the bio-plastics and the petro-plastics [10].

1. Production Of TPS

There are several traditional processing technologies to manufacture TPS such as; film casting, compression moulding, injection moulding, and extrusion. Solution film casting technology is an easy method for producing starch-based films. The efficiency of this method is low, therefore, it is not utilised in industrial scale production. All technologies begin with separation of starch from their raw materials (eg. potato). The separated starch is further used alone or blended with other polymers, fibres or fillers. In compression moulding technology, the starch mixture is then prepared by thoroughly mixing the following components together in varied amounts: 1) starch, 2) water, and 3) plasticiser. In case the addition of reinforcing bio-fibres or bio-fillers are required, they are cleaned, cut, chemically and mechanically treated, dried and blended gradually with the starch mixture. In order to end up with high quality plastics, the amounts of each component and the reinforced materials should be carefully determined by conducting preliminary trials. Following that, the blend is placed in the mould cavity and pressed at certain pressure, temperature and time [103-105]. Moreover, the use of plasticiser helps in increasing the flexibility and applicability of the plastic [96]. High or excessive amount of plasticiser lead to a reduction in the tensile strength of plasticised films by subsequently making the hydrogen bonds between starch chains weak [106]. During processing of TPS, other additives can be added to the plastic for various purposes. For instance, to reduce the possibility of the sample to stick to the die, lubricants such as; magnesium stearate, calcium stearate and fluoro-elastomers can be used [105]. To testify the quality and properties of the plastic produced according to an international standards, several common testing standards can be applied such as; American Society for Testing and Materials (ASTM) [100].

2. Reinforcement Materials

Due to the abundant availability, low cost and the thermal behaviour of starch and as it is renewable and biodegradable, it has been considered as one of the most promising raw materials [107]. Despite that, starch is still not completely favourable in some industrial purposes because of the poor mechanical properties, the brittleness and the high sensitivity to moisture. Therefore, the production of TPS is projected to be less than 5% of the total bio-plastic produced in 2019 which is expected to be approximately 7.8 million tons. Recently, attention towards

improving the properties of the TPS have increased, For example, the blending of TPS with polymers, fillers or fibres as reinforced material are the most recommended solution by many studies. Many researchers have blended TPS with other petroleum polymers such as: PE, PP and PS to enhance the properties and produce polymers for long term applications. The studies have resulted to TPS with better properties but the problem behind that is the plastics produced are not green. Furthermore, the incorporation of variety of natural reinforced materials with TPS have been investigated and varied improvement in the properties have achieved. Despite that, discovering and studying of more natural reinforced materials are still required [108, 109].

In 2016, a study assessed the thermal, tensile, and physical properties of TPS after blending it with Agar derived from marine algae [110]. Sugar palm starch was used in the study as a raw material. After preparing, plasticising and blending the starch with agar, TPS was produced through thermo-compression moulding process. Several techniques and tests such as; FTIR, DSC, SEM, moisture absorption and thickness swelling test and tensile test have been applied to investigate the quality and properties of the TPS produced. Remarkable improvements in thermal and tensile properties were achieved after the addition of agar (see Figure 6, Figure 7 and Table 3). The study pointed out that, the material produced is a promising eco-friendly plastic and most suitable for short life applications like packaging container, tray, etc. [110].

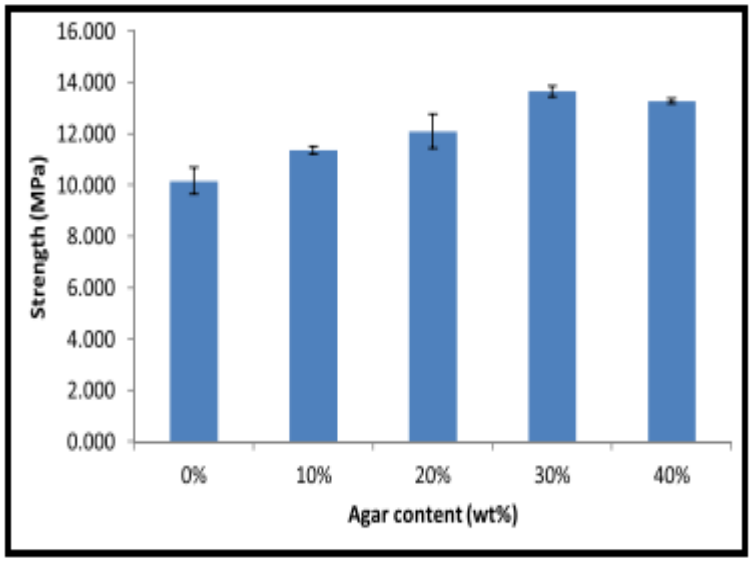


Figure 6: The influence of the Agar on the tensile strength of the thermoplastic SPS/Agar [110].

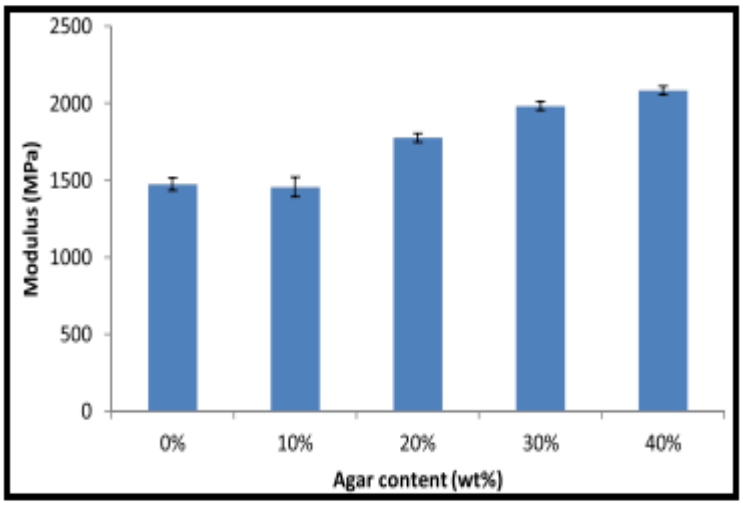


Figure 7: The influence of the Agar on the tensile modulus of the thermoplastic SPS/Agar [110].

Table 3: Glass transition, Tg and melting temperture of thethermoplastic SPS/Agar [110].

Samples	T_g (°C)	T_m (°C)
0% Agar	137.9	172.2
10% Agar	151.1	178.9
20% Agar	152.3	192.1
30% Agar	156.5	219.8
40% Agar	154.5	218.7
Native SPS	140.7	171.3
Native Agar	152	186.6

In addition, the authors [110] expanded the research prescribed above in 2017 to investigate the mechanical, thermal and biodegradation properties of the TPS/agar in which has been produced in the previous study after blending it with a seaweed waste called Eucheuma cottonii as a bio-filler. The study has investigated the properties of the TPS/Agar after incorporation it with five concentrations of the seaweed ranging from 0 to 40% [103]. Significant improvements have been observed in the tensile, flexural strength, impact strength properties and thermal stability of the TPSA/seaweed composites (see Figure 8 and Figure 9). The Scanning Electron Microscopy (SEM) of the tensile fracture surface of TPSA/ seaweed showed a homogeneous structure of the composite with no apparent phase separation was observed (Figure 10). That is indicated to a good compatibility of all components in the matrix. This compatibility probably occurred due to some reasons, with one of those reasons been; the seaweed and the TPSA matrix contain similar hydrophilic behaviour. Therefore, good adhesion between the matrix and the seaweed was achieved and resulted in a more homogenous surface [103].

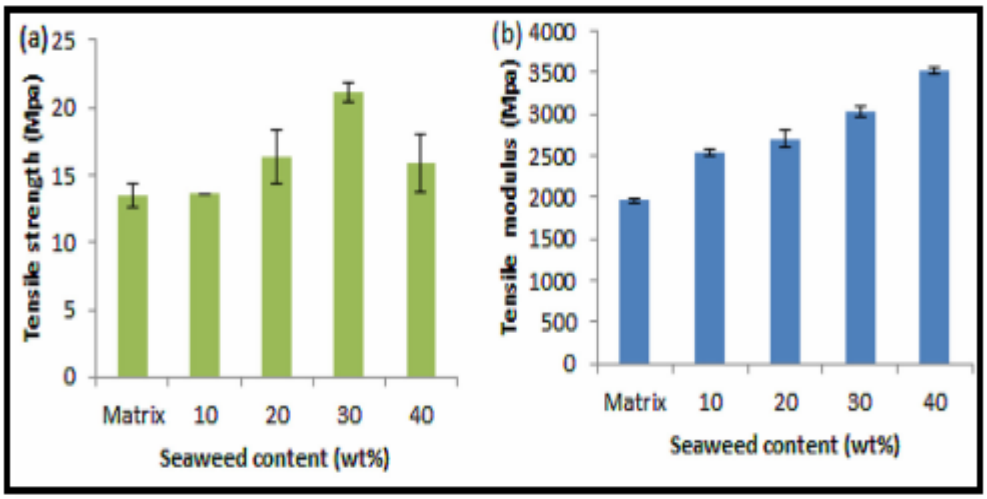


Figure 8: Tensile properties of the TPSA/seaweeds composites (a) tensile strength, (b) Tensile modulus [103].

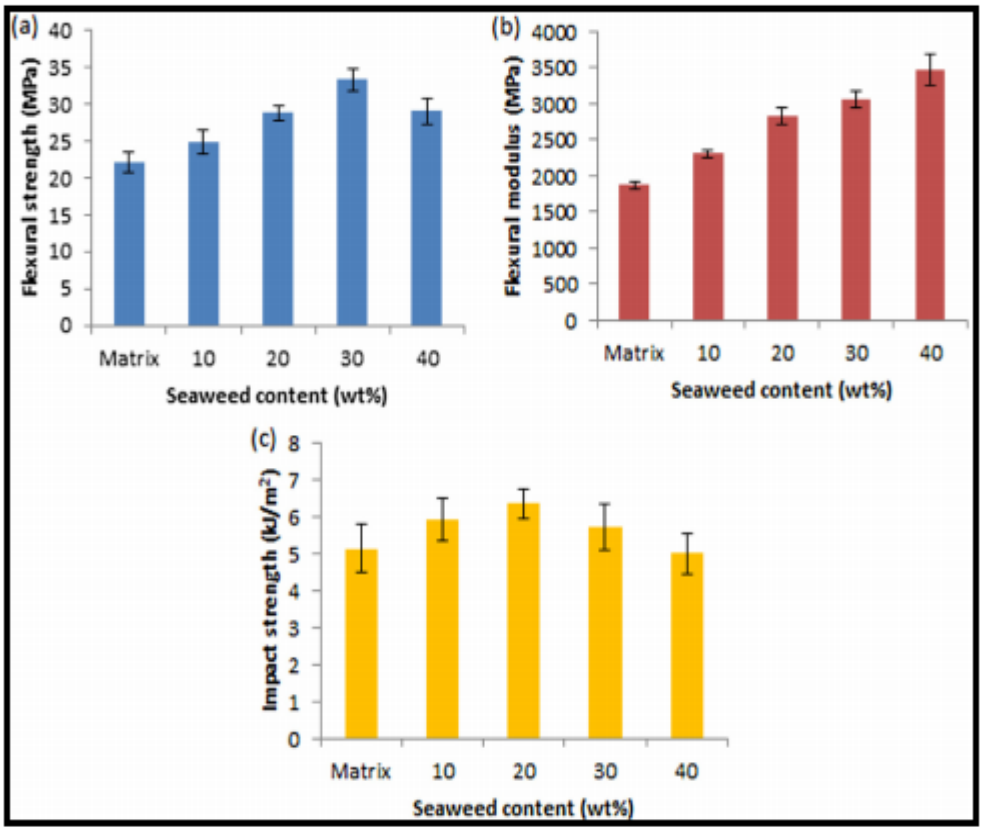


Figure 9: Flexural and impact strengths properties of the TPSA/seaweeds composites (a) flexural strength, (b) flexural modulus and (c) impact strength [54].

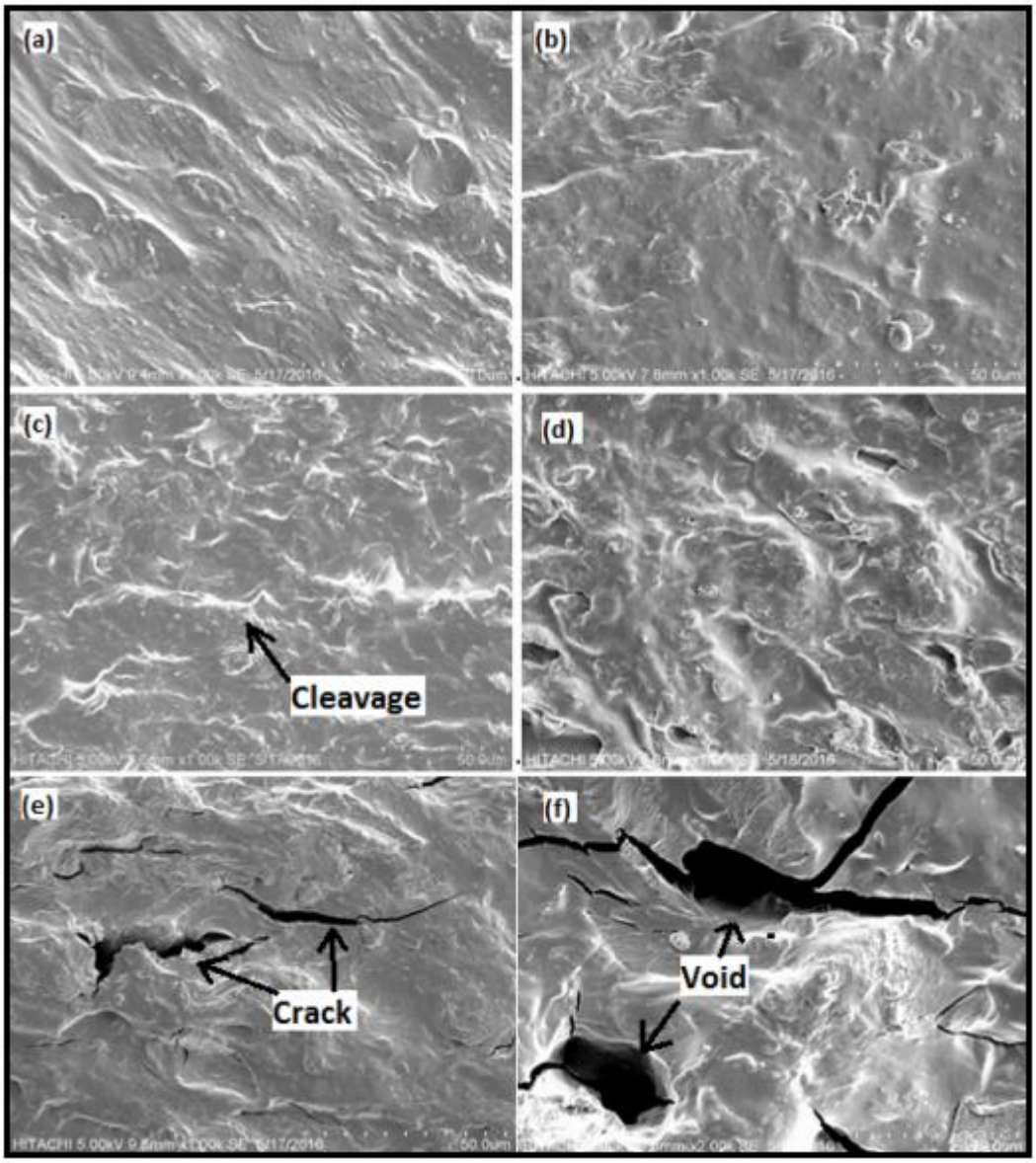


Figure 10: SEM investigation of tensile fracture surface of TPSA/seaweed composites (a) TPSA matrix (b) 10% seaweed (c) 20% seaweed (d) 30% seaweed (e) 40% seaweed composites and (f) void in TPSA/seaweed composites [103].

According to Tadini, C. C. [109], the incorporation of starch and natural fibre as reinforced materials from the same source where the starch isolated to produce reinforced TPS is a promising composite materials, due to the natural affinity of the fibre and the seed (where the starch originated). Castaño, J. et al. [111], carried out a study in 2012 to evaluate the blending of pehuen cellulosic husk as reinforced material with the thermoplastic pehuen starch. The study concluded, enhancing that the natural affinity between the fibre and starch from the same source led to an improvement in some vital properties of the composite such as; mechanical

properties, thermal stability and the homogeneity between the enhancer material and the plasticised starch.

2.3 Characteristics Of Some Starchy Fruits And Vegetables

2.3.1 Potato

Potato is very common vegetable all over the world and represents the fourth main crop after rice, wheat and maize. It is highly consumed in people's daily lives and considered as a good source of energy. It is processed, cooked, baked, boiled, or fried and served in multiple forms such as; chips, mashed potatoes, potato pancakes, potato dumplings, etc. Total dry matter of fresh potato contains approximately 81–82% total digestible nutrient (TDN), about 10% protein and very low amount of fibre [112]. However, potato processing industries, especially potato chips industries leave a huge quantity of potato residues after potato processing. These residues are causing environmental issues due to their microbial spoilage. Usually, prior processing potato, potato peels are removed [113]. According to Chang K.C. [114], potato processing companies generates about (70,000 - 140,000 tons/ year) potato peels worldwide. Potato peels as a biomass have proved its significant potential in generating various bio-fuels and bio-products including; biogas, bio-ethanol and bio-plastic. The peels can also be extensively found using as fertiliser or low value animal feed. Aboderheeba, A. [32], evaluated the production of biogas from mechanically pre-treated potato peels. The study achieved significant biogas yield. Achinas, S. et al. [115], examined the performance of the AD of potato peels in different inoculum-to-substrate ratios. The study revealed that, the co-digestion of potato peel and cow manure yielded up to 237.4 mL CH_4/g-VS. In addition, a maximum CH_4 of 217.8 mL CH_4/g-VS was yielded from the mono-digestion of potato peels. However, potato peel is rich in pectin, cellulose and starch. According to Mahmood A. [116], potato peel and trimmings are the wastes of potatoes and constituted approximately 12% of the potato fresh weight. Potato wastes contain approximately 66.78% of their dry weights as a starch, 3.39% pectin, cellulose 2.20 %, 14.70% crude protein and components as well in different proportions.

As is well known, starch can be obtained from many traditional sources such as; potato, maize, and cassava and so on. Starch properties and concentrations varied from one source to another. Potato starch concentration and properties varied

according to each potato type. A comparative study of the properties of starch from Irish potato (Solanum tuberosum) and sweet potato (Ipomea batatas) grown in Nigeria, revealed that, the starch granules of Irish potato are larger and have wider range of size distribution than sweet potato. Significant differences between them have been noticed in regard to moisture, nitrogen, fat, phosphorus, and Amylose contents of the starch. Sweet potato starch have higher gelatinisation peak temperature than Irish starch. In addition, sweet starch paste has lower paste clarity and higher retro-gradation than Irish potato [117]. However, potato starch granules normally consist of 20-30% Amylose and 70-80% Amylopectin [118]. In regards to the physical properties, potato starch is unique amongst the other starch types (e.g. cereal types) due to a number of factors including; particle size, purity, relatively long Amylose and Amylopectin chain lengths, existence of phosphate ester groups on Amylopectin and, ability to form thick visco-elastic gels upon heating and subsequent cooling [119]. In addition to that, one of the major properties in which made potato starch is widely utilised in many industrial applications is its large capacity to form very clear gels when gelatinised [120, 121].

2.3.2 Mango

Mango is a common tropical fruit rich in nutrients. It is composed of a number of useful nutrients including: water, minerals, vitamins, fibres and antioxidants in which make it as one of the most consumed fruits in the worldwide. In term of consumption, mango is the fifth most consumed fruit after citrus, banana, grape and apple [122]. In addition, mango has some special features such as; distinctiveness and multiple flavours, its nutritional quality and its numerous industrial derivatives and applications. On the other hand, mango is the second most produced fruit in the world. An estimation study has indicated that, the production of mango has increased from about 10 million tons in 1960 to 35 million tons in 2008 [123]. According to a projection in 2008, Asia represents as the largest continent in the production of mango at the rate of 75% of the total [122, 123]. In 2015 [124], the estimated production of mango was at 42 million tons annually. Where, India was the largest producer with a production at 1,525,000 tons annually [124, 125]. While, Europe represents only to 0.02% of the production of mango [125].

Furthermore, mango is normally consumed fresh. Mango processing industries consume only 1% of the total mango produced [126, 127]. Mango fruit primarily consists of three parts: edible tissues (33-85% of the total fruit), peel (7-24%) and kernel (9-40%) [51]. Whether processed or fresh, mango peel and kernel are disposed and considered as residues. Based on a projection [128], 35-60% of the total fruit are discarded after processing. According to Leanpolchareanchai, J. et al. [129] in 2014, approximately one million tons of mango seeds as residue are generated per year and not used in any commercial applications.

Moreover, mango peels are rich source of many bioactive compounds and enzymes, such as; protease, peroxidase, polyphenol oxidase. In dry weight basis, it contains about 20.8–28.2% of carbohydrate [130]. While, mango kernel is composed of multi compounds such as; starch, fat, flour and antioxidant [131, 132]. Due to the increase in the production of mango worldwide and the huge amounts of residues generated after processing of the fruit, the attentions towards the utilisation of the wastes in several applications has been increased. This huge source of waste would contribute in a serious environmental problem if not handling or utilised properly [133, 134]. However, mango seed contains approximately 21% starch [135]. The properties of this starch is quite similar to Tapioca starch. In comparison with other common starch types, the viscosity of pure mango seed starch is a little bit lower. However, its solubility is higher [135]. The proportions of Amylose and Amylopectin in the starch are 39.9% and 60.1% respectively [136]. Nilani, P. et al. [137], carried out a study on the development of polysaccharide based biopolymer films, and found that mango kernel has similar characteristics comparable to conventional polymer films from waste materials of plant. The study concluded that, the mango kernel starch is a suitable raw material for the production of natural polymer. In addition, various bio-energy production studies have used mango residues as a biomass for productions of several bio-energy i.e. biogas [138].

Furthermore, a literature survey [139] revealed that only few studies have been published yet to utilise this low-cost starch in industrial applications. Recently, multiple studies have assessed the potential of using Chaunsa mango kernel starch as a coating for enhancing shelf- life of vegetable and as composite films when it is

blended with gums. The studies suggested using mango kernel starch in food packaging industries as a film or coating. [139, 140]

2.3.3 Avocado

Avocado is a single seed and one of the most important tropical fruit all over the world. It belongs to the Lauraceae family [141]. The total mass of unpeeled avocado with medium or thick skin is ranging from 140 to 400g. When ripe, avocado turns into purplish black. Avocado has a pear shaped, oval or round with a short neck. It has a length between 7.7 cm to 33 cm and is 15 cm in width. Avocado's colour is varied from one type to another such as; yellowish green, dark green or reddish-purple. It has bittery or nut-like flavour. The edible part of avocado is the pulp and flesh. The single seed of avocado can be oval, round or oblong shaped and located in the centre of the fruit. The seed length ranges between 5 to 6.5 cm and covered with brown thin coat. Moreover, avocado originated from Mexico and Latin America, and Mexico is the largest producer of avocado in the world [142]. In 2011, the quantity of avocado produced in Mexico was 337,977 tons, in which represents 47% of the world production [142, 143]. United Kingdom is the third largest importer of avocados in Europe. In 2008, UK imported 11,753 tons of avocado from South Africa [144]. Furthermore, Hass is very popular type of avocado in the U.S. In comparison with other types, Hass has longer shelf life and bigger fruit size. Due to that, and because of its rich nut-like flavour, Hass is the most favourite and consumed type of avocado and dominant on the global avocado market [142, 145].

Avocado is composed of high nutrients including, vitamins, minerals, potassium, phosphorus, magnesium, iron and bioactive phytochemicals [142, 146]. The concentration of the nutrients is also varied from a type to another depending on number of factors. Avocado flesh and pulp are high in lipid which ranges from 3% - 30% of the total fresh weight [147].

It is possible to find avocado fruit, used in various applications such as; cosmetics, and livestock feed, however, the main uses of it is for human consumption. Avocado seeds and peels are not practically used and often disposed of by land-filling. Avocado seed accounts for 26% of the total fruit mass [148]. It contains 80.1% starch [149]. Despite that, the seeds are not used for animal feed as

it contains of high concentration of polyphenols in which has very bitter taste and can be toxic at high doses [148, 149]. A study on the avocado starch revealed that, the starch content is depending on the cultivar and can reach up to 74.47% of the dry weight [53]. According to Chel-Guerrero L. et al. [141], the Amylose content of avocado seed starch is 15-16%, while the solubility of starch, swelling power, and water absorption capacity are 19-20%, 28-30 g water/g starch and 22-24 g water/g starch respectively. In the study, the authors revealed that, the seed starch has a potential to be used as raw material for the production of biodegradable polymers. On the other hand, avocado peels and seeds have been used in several studies as single substrate or mixed with other substrates to produce bio-fuel i.e. biogas and bio-ethanol [138]. A study in 2010 [150], compared between the properties of a starch isolated from avocado and starch isolated from maize. The study revealed that, the swelling, moisture uptake and paste clarity of avocado starch are less than that of the maize starch. Both (T_g) and gelatinisation temperatures of avocado starch are higher than that of maize starch. While, the (T_m), ash value and the various densities of avocado starch are similar to that of maize starch. Lastly, the study concluded that, avocado starch has distinct physico-chemical and binder properties with some similarities to the standard maize starch.

2.4 Renewable And Non-Renewable Energy

2.4.1 Energy Demand

People have become more dependent on energy than ever before. Nowadays, energy is used almost everywhere in many forms such as; chemical, mechanical, light, electrical, potential, kinetic, heat, nuclear, and many more. In modern times, people have become fully dependent on energy and the demand for it is continuously increasing [18].

According to the international energy agency IEA 2013 [151], the total global primary demand for energy increased from 5,536 million tons of oil equivalent in year 1971 to 10,345 million tons in year 2002. Which indicates to an average annual increase of 2% and about the double in 30 years. By 2008, the demand had increased to 12,271 million tons indicating an average annual increase of approximately 3%. This increase of about 50% in the average annual rate was due to the rapid growing of energy demand in China in particular and Asia pacific.

From 2008 to 2011, the annual increase rate in energy demand decreased to 2.1%. That's decrease was because of the deep recession in the United States and Europe as well where the energy uses came down. Table 4 shows clearly the world energy demand for different energy sources and the annual changes for the period from 1971 to 2011. [151]

Table 4: World primary energy demand by fuel (Mtoe) [151].

Energy source/ type	1971	2002	2008	2011	Annual change 1971-2002 (%)	Annual change 2002-2008 (%)	Annual change 2008-2011 (%)
Coal	1,407	2,389	3,315	3,773	1.7	5.6	4.4
Oil	2,413	3,676	4,059	4,108	1.4	1.67	0.4
Gas	892	2,190	2596	2,787	2.9	2.88	2.4
Nuclear	29	892	712	674	11.6	-3.7	-1.8
Hydro	104	224	276	300	2.5	3.6	2.8
Biomass and waste	687	1,119	1,225	1300	1.6	1.6	2
Other renewable	4	55	89	127	8.8	8.46	12.6
Total	5,536	10,345	12,271	13,069	2.0	2.9	2.1

In 2019, Global energy demand increased by 0.9% of the World energy demand growth rate. This increase is equivalent to 120 million tonnes (Mtoe) of oil and only 40% of the growth rate observed in 2018. This deceleration is primarily attributed to, the slower global economic growth and the impact of milder weather on heating and cooling [152]. Figure 11 shows World energy demand growth rate in the period of 2011-2019. Due to the pandemic of the COVID-19 virus and as a consequence of the efforts to slow the spread of the virus, Global energy demand decreased by 3.8% in the first quarter of 2020 [153].

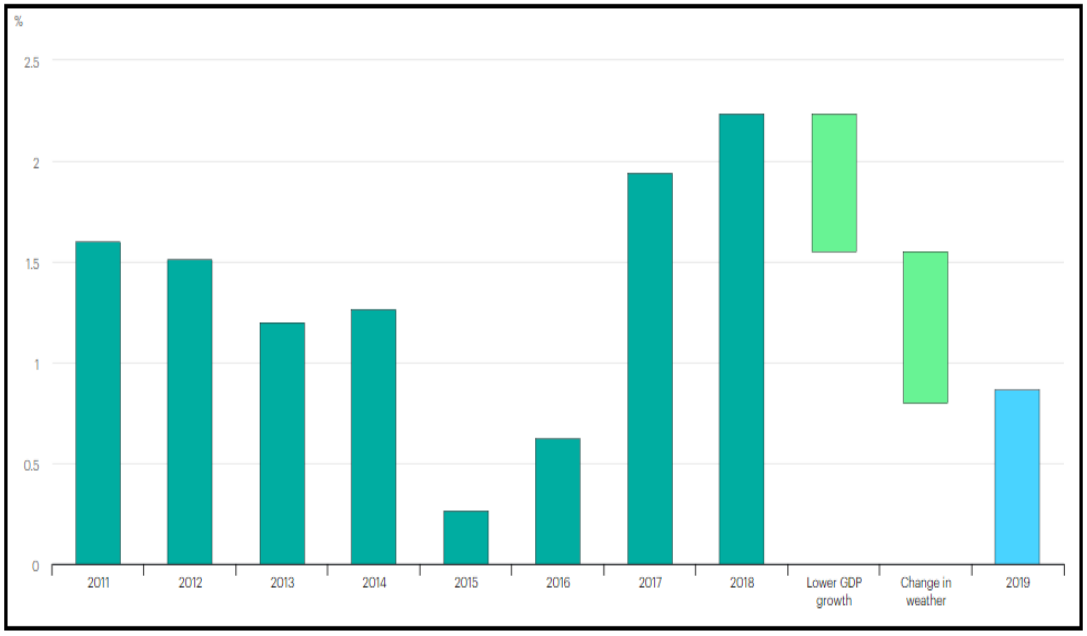


Figure 11: World energy demand growth rate in the period of 2011-2019 [152].

Energy plays a very important role in the economic and social development of any country. Not only that, but it also has a great impact on improving the quality of life [18, 154]. Furthermore, the demand for energy increases rapidly, and energy demand is expected to rise by 1.3% each year to 2040. That is expected to happen if the world continues along its present path, without any additional changes in policy [155]. Due to the environmental impacts of energy such as; pollution and global warming, and other reasons as well, the need to find another cheap, renewable, sustainable, and environmentally friendly energy sources is growing rapidly.

2.4.2 Energy Classification

Energy in all its forms can be classified into two categories: renewable and non-renewable energy. Most of the energy used in the world nowadays are from non-renewable energy supplies. International energy outlook 2017 (IEO2016) have estimated that, the world renewable energy consumption will grow at rate of 2.3% per annum between 2015 and 2040 [156]. The next few sections briefly described the two categories as well as some of their forms.

2.4.3 Non-Renewable Energy

Non-renewable energy is the energy created by burning fossil fuels such as crude oil. The majority of the developed countries still rely on non-renewable energy sources. Crude oil, natural gas, and coal are some types of fossil fuels.

The processes whereby fossil fuels are formed, such as; burying, and subsequent pressure and heating, of dead plant and animal matter or organic matter, over millions of years. The trapped energy within these fossil fuels is released and used when the fuels are burnt.

One of the major disadvantage of using non-renewable energy is the question of when the fossil fuels will deplete? Additionally, they are non-renewable, pollute the environment, and burning fossil fuels produces greenhouse gases which include a high amount of carbon dioxide that contribute significantly to global warming. [187]

1. Main Types Of Fossil Fuels

Fossil fuel consists of three main types: crude oil, coal, and natural gas. In order to meet the future need of these three types of fossil fuel in 2050 and later, it is crucial to note their available reserves. According to the statistical review of the BP company of world energy (June 2016) [157], the total world proved of each of these type are shown in Table 5:

Table 5: Total world proved reserves at the end of 2015 for each fossil fuel type and R/P ratio [157].

Fossil fuel type	Total global proved reserves at the end of 2015	Reserves to production ratio (R/P)
Crude Oil	1697.6 billion barrels	50.7 years
Natural Gas	186.9 trillion cubic meters (tcm)	52.8 years
Coal	891531 billion tons	114 years

2.4.4 Renewable Energy

Renewable energy sources does not pollute the environment [158]. It is the best and only choice for sustainable economic growth, for harmony between humans and the environment, and for sustainable development [159]. Renewable energy sources are clean sources of energy, unlike fossil fuels, are continuously replenished, and will not run out. These sustainable or natural sources are primarily the sun (solar energy), wind, moving water (hydropower, wave and tidal energy), heat below the surface of the earth (geothermal energy), and biomass (wood, waste, energy crops, etc.) [48]. According to the Sustainable Energy Authority of Ireland (SEAI) [160], during 2018, the total renewable energy increased by 10.2% and reached 1,471 ktoe. Hydro and wind grew by 0.4% and 16% respectively. Biomass use increased by

approximately 11% in 2018, to 410 ktoe, and other renewables dropped by 2.8%, to 259 ktoe. Additionally, the overall share of renewables in primary energy stood at 10.0% in 2018, up from 9.3% in 2017 [160].

Moreover, the world production of electricity derived from various renewable energy sources is projected to grow 2.7 times from the year 2010 to 2035. While the consumption of bio-fuels is expected to sharply increase up from 1.3 million barrels of oil per day in 2010 to reach 4.5 million barrels of oil per day in 2035. That implies to an increase of more than triple times in the consumption of bio-fuels over 25 years. In the same period, the production of heat from renewable energy sources is also expected to increase from 337 to 604 Mtoe. [48]

1. Biomass As Renewable Energy Source

Most biomass energy sources are available almost everywhere. They range from wood, wood wastes, agricultural crops and their waste by-products, and municipal solid waste, to animal wastes, waste from food processing, seaweeds and algae. Furthermore, according to a previous projection, the technical potential for biomass is projected in the literature to be probably as much as approximately 1500 EJ/year by 2050 [161]. Estimates of the world primary bio-energy potentials available around 2050 published in the last years span range from 30 to more than 1300 EJ/year [162, 163]**.**

In addition, biomass sources cover a wide range of materials that can be used for energy production, and can be categorised in two ways:

1) Modern biomass: aims to be a substitute for conventional energy sources - Involves large-scale use.

2) Traditional biomass: confined to developing countries - Involves small-scale use [162, 164].

Biomass contains various proportions of Cellulose, Hemicellulose, Lignin and a small amount of extractive. Cellulose usually represents the largest fraction of biomass (from 40% to 50% by weight) followed by Hemicellulose (20% to 40%) [164, 165]. For example, the sugarcane bagasse consists of about 40–50% Cellulose, 20–30% Hemicellulose, 20–25% Lignin and 1.5–3.0% ash [18].

In the development of the process for producing derived fuel and chemicals, chemical structure of the biomass and the main organic components of it plays an important role. Furthermore, the size of a biomass particle defines the importance of a specific type of biomass which relies on different properties such as; chemical and physical properties [166].

Biomass is possibly the oldest source of energy. After coal, petroleum and natural gas, biomass is the fourth largest primary energy resource worldwide. The total amount of biomass which a plant produces depends mainly on two major factors which are:

a) The amount of solar energy that the plant absorbs.
b) The amount that can be stored as carbohydrates.

For photosynthesis process, plants use 0.1% of solar radiation. This can be represented by the following Equation 1 [18]:

$$CO_2 + H_2O + \text{light} \xrightarrow{\text{Chlorophy II}} CH_2O + O_2 \qquad \text{Eqn.1}$$

Photosynthesis involves CO_2, water, and chlorophyll, and produces carbohydrates which makes up the largest part of plants tissues. Photosynthesis traps a certain amount of solar energy in chemical bonds and uses this to produce carbohydrates [164, 167].

There are several types of conversion processes for converting biomass into biomass-based energy fuel. Bio-chemical conversion is one of the few processes that are environmentally friendly in the way they produce energy from biomass. However, conversion of biomass into useful form depends on the type, properties and quantity of feedstock, the desired form of the energy, environmental standards, economic conditions, and project-specific factors [18]. Moreover, biomass as an bio-energy source has many advantages and can be converted into three major kinds of products: electrical or heat energy, fuel for transportation such as; biodiesel, and feedstock for chemicals [164-167]. On the other hand, biological conversion of biomass is conducted by alcoholic fermentation technique to produce liquid fuels, and anaerobic digestion (AD), or fermentation, results in biogas. The most common

and suitable types of biomass that are used in AD are high moisture herbaceous plants such as vegetables, sugar cane, sugar beet, corn, seaweeds, and manure [168].

Additionally, the largest advantage for the biomass as a bio-energy sources is its relatively straightforward transformation into transportation fuel. As the bio-fuel does not require more infrastructure development, its development is being viewed very favourable by the majority of countries in the world. Bio-fuel and other transportation means like; electric vehicles are greatly helping in diversity the fuel base for future transportation [169]. According to (IEA 2019) [170], Figure 12 depicts the bio-fuels production growth by country/region in the period of 2013-2018 and the projected growth in the period of 2019-2024.

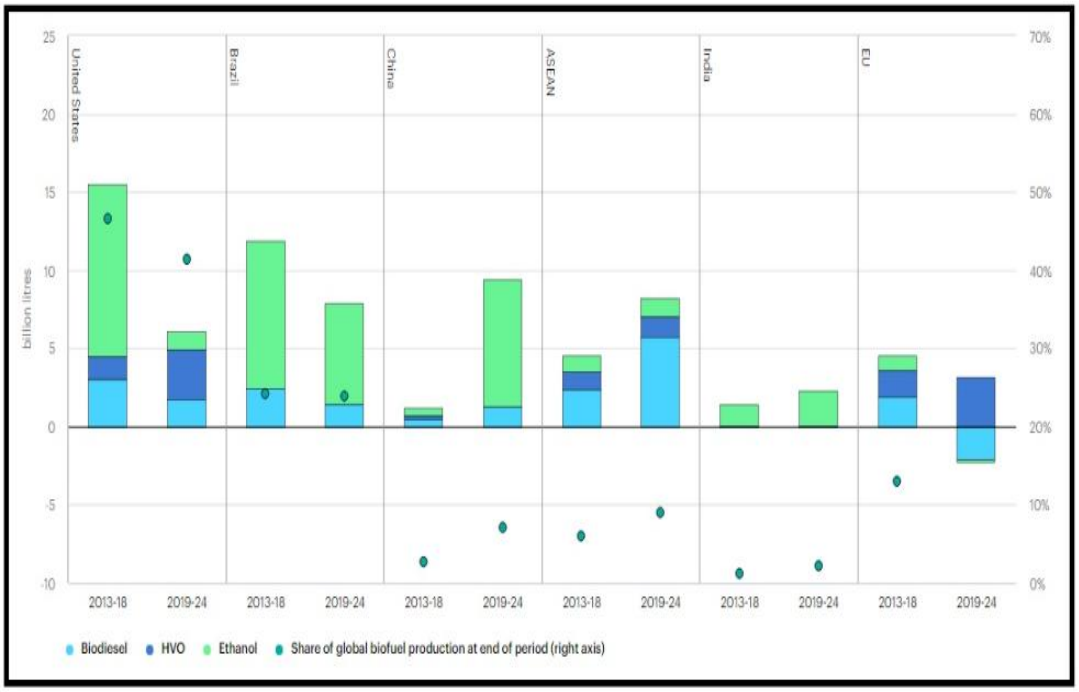


Figure 12: The bio-fuels production growth by country/region in (2013-2018 and 2019-2024) [170].

As shown in the above figure, China is projected to have the highest bio-fuel production growth compared to any other country. While, Brazil registers the second-highest growth. The U.S and Brazil are still projected to provide two-thirds of total bio-fuel production in 2024 [170]. Bio-fuel and some of its energy forms are discussed in more detail in this chapter.

2.4.5 The Challenges Of Renewable And Non-Renewable Energy

Burning of non-renewable energy sources has resulted in the release of a large amounts of carbon dioxide into the atmosphere. Non-renewable energy is significantly contributing to the sharp increase in greenhouse gases. According to many studies, as a result of burning of fossil fuels, the earth's temperature has been gradually increasing over the past decade. The amount of fossil fuels is limited and will be depleted one day. The emissions from power plants, factories, and so on, have contributed to the phenomena of global warming that represents a serious threat to the world [48, 49, 171]. As a consequences of the efforts to slow the spread of the COVID-19 pandemic, the analysis of daily data of the International Energy Agency (IEA) through mid-April 2020 shows that, countries in full lockdown experienced an average 25% reduction in energy demand per week and countries in partial lockdown an average of 18% decline [153]. Due to that too, daily global CO_2 emissions declined by –17% (–11 to –25% for ±1σ) by early April 2020. At their peak, emissions in individual countries declined by –26% on average [172].

However, most non-renewable energy sources, are very efficient in generating power. Most machines, devices, transportations means, and so on, are powered by non-renewable energy.

On the other hand, renewable energy is the required alternative to non-renewable energy as it is available everywhere in the world, environmentally friendly and would significantly reduce air and environmental pollution. But, the question should be asked then is, will biomass be as efficient as non-renewable energy sources in generating energy? In comparison, renewable energy has low energy densities than fossil fuels. In other words, a significantly greater volume of biomass fuel is required to produce the same energy as a smaller volume of fossil fuel. In addition to the advantages of renewable energy, it can be easily regenerated and the maintenance cost required to install and use the renewable energy is relatively cheap [48, 49, 171, 173].

2.4.6 Bio-Fuel Overview

According to the Food and Agricultural Organization (FAO) definition [174], bio-fuels are “fuels produced directly or indirectly from biomass such as fuelwood, charcoal, bio-ethanol, biodiesel, biogas (methane) or bio-hydrogen”. While,

biomass is “the material of biological origin excluding material embedded in geological formations and transformed to fossil, such as energy crops, agricultural and forestry wastes and by-products, manure or microbial biomass” [174]. Biogas as a bio-fuel, are discussed in more detail in this chapter.

However, due to the global demand to substitute fossil fuels, the interests of bio-fuel by many developed and developing countries have increased rapidly in the last few decades [175, 176]. Some countries such as the USA and EU countries, have set plans to replace a specific percentage of the transportation fuel into bio-fuel [177, 178]. After adoption of the sustainable development targets in September 2015, most countries now should set such a plan [179].

Bio-fuels basically comes in three forms; solid, liquid and gaseous fuels derived from organic matter. In general, they are classified into two main categories: primary and secondary bio-fuels. The primary bio-fuels are used in an unprocessed form, commonly for heating, cooking or electricity production such as; fuelwood, wood chips and etc. While, secondary bio-fuels are generated by the processing of biomass, for example ethanol, biodiesel, and so on, which can be used in vehicles and different industrial processes [180]. Depending on the feedstock and conversion technology used for their production, secondary bio-fuels are further divided into first, second, third and fourth generation of bio-fuels [181]. Table 6 shows this bio-fuel classification.

Table 6: Bio-fuel classification [180].

Bio-fuel classification		Bio-fuel and conversion technology	Substrate
Primary			fuelwood, wood chips, pellets, animal waste, forest and crop residues, landfill gas
Secondary	1st generation	▪ Bio-ethanol by fermentation ▪ Bio-diesel by transesterification	Seeds, grains, sugars and vegetable oils
	2nd generation	▪ Bio-ethanol by enzymatic hydrolysis ▪ Bio-methane by Anaerobic Digestion AD ▪ Methanol, Fischer-Tropsch gasoline and diesel, mixed alcohol, dimethyl ether and green diesel by thermo-chemical processes	Lignocellulosic biomass, agricultural wastes, municipal and industrial wastes
	3rd generation	▪ Bio-diesel by transesterification ▪ Bio-ethanol by fermentation ▪ Bio-hydrogen by fermentation ▪ Bio-methane by AD	Microalgae (unicellular microorganisms), macroalgae (seaweeds)
	4th generation		Microalgae (unicellular microorganisms), macroalgae (seaweeds)

Bio-fuels offers promising fulfil of a number of benefit related to major aspects like: energy security, economics, and the environment. In order to meet these benefits, several obstacles must be overcome [180]. Table 7 illustrates these benefits and the challenges to realise them.

Table 7: Potential benefits and challenges or obstacles of bio-fuel [180].

Benefits	Challenge
Energy security ▪ Domestic energy source ▪ Local distributed ▪ Well connected supply-demand chain ▪ High reliability	Feedstock ▪ Collection network ▪ Storage facilities ▪ Food-fuel competition
Economic stability ▪ Price stability ▪ Employment generation ▪ Rural development ▪ Reduce inter-fuels competition ▪ Reduce demand-supply gap ▪ Open new industrial dimensions ▪ Control on monopoly of fossil rich states.	Technology ▪ Pre-treatment ▪ Enzyme production ▪ Efficiency improvement ▪ Technology cost ▪ Production of value added co-products
Environment ▪ Better waste stabilisation ▪ Reduce local pollution ▪ Reduce GHGs emissions from energy consumption ▪ Reduction in landfill sites	Policy ▪ Land use change ▪ Fund for research and development ▪ Pilot scale demonstration ▪ Commercial scale deployment ▪ Policy for bio-fuels ▪ Procurement of subsidies on bio-fuels production ▪ Tax credit on production and utilisation of bio-fuels

The First generation of bio-fuel are broadly produced from food crops. Its production process is carried out by abstracting oils for use in bio-diesel or producing bio-ethanol through a fermentation process. However, the three major bio-fuels from the first-generation list which are commercially utilised, are: biodiesel, bio-ethanol and biogas. However, there are many debates over this generation as it is produced directly from food crops, especially as there are around one billion of people in the world who are suffering from famine. Another major disadvantages of the first generation are: higher production cost, several bio-fuels can produce negative net energy gains, and they are emitting more carbon during the production process compared to the amount of carbon their feedstock are capturing in their growth stages [180, 182-184]. The European commission opened a debate in 2009 in order to reduce first generation food-based bio-fuels in favour of the second generation of bio-fuel (advanced bio-fuels). While, the primary cause beyond that was to limit the uncertainty in estimations of the impacts of indirect land use change emissions (ILUC) of food-based bio-fuels [185, 186].

The Second generation of bio-fuel, is mainly produced from non-food feedstock such as; wood, organic waste, food crop waste and specific biomass crops. This generation has overcame the limitations of the first generation bio-fuels as the feedstock used in manufacturing of second generation bio-fuels are normally not food crops. For example, waste vegetable oil is considered as second-generation bio-fuels because it has already been used and is no more valid for human consumption. Whilst, virgin vegetable oil is attributed to the first generation of bio-fuel. Moreover, as a second generation is produced from variety feedstocks, various conversion technologies are required in extracting energy from them. Second generation conversion technologies are classified also into bio-chemical and thermo-chemical technology. Some of the thermo-chemical conversion technologies that are used nowadays are: gasification, pyrolysis and torre-faction [180, 184]. In terms of the production of second generation bio-fuels using biochemical technology, numerous biological and chemical processes are being adapted. Fermentation processes with unique or genetically modified bacteria is widely common for second generation feedstock such as: municipal waste [180, 182, 184].

The Third generation of bio-fuel is basically generated from aquatic cultivated feedstock such as; algae, microbes and microalgae used in this generation are considered the applicable alternative energy source that is free of the major drawbacks attributed to the first and second generation of bio-fuels. However, algae as a feedstock has the advantage that it is low-cost, high-energy and entirely renewable feedstock. It has also the potential to ensure big growth yields without requiring arable land, high percentage of carbon store during photosynthesis and a negligible or low amount of lignin makes them less resistant to degradation than lignocellulosic feedstock, avoiding the demand for energy-intensive pre-treatment processes [168, 187-190]. Specifically, macro-algae, commonly known as seaweeds. Anaerobic digestion process (AD) as a biological conversion process is the most direct approach to produce bio-fuel form macroalgae [187].

The Fourth generation of bio-fuel is quite similar to the third generation. It is produced from aquatic cultivated feedstock as well. It is different from third generation as it is aimed not just to produce bio-fuel but to capture and store CO_2 as well. Additionally, a review research in 2011 [191], concluded that 4^{th} generation

bio-fuel production has introduced the "cell factory" concept in this field, and shifted the research paradigm. There are still remains various technical bottlenecks in algae bio-fuel research and development. Those bottlenecks can only be solved by the use of post-genome tools on these photosynthetic organisms. Figure 13 illustrates the 4th generation bio-fuel production process.

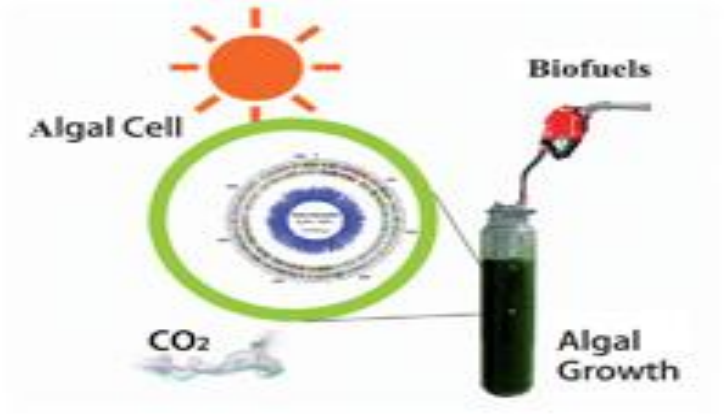


Figure 13: 4th generation producing bio-fuel from engineered algae [191].

2.5 Anaerobic Digestion (AD)

Anaerobic digestion (AD) is a biological process in which volatile organic materials are broken down in the absence of oxygen and the presence of anaerobic micro-organisms to generate a gas. Generally, AD involves the degradation and stabilisation of organic materials in the absence of oxygen by microbial organisms and results in the formation of gas and microbial biomass. The energy content of biogas from AD is chemically bound in methane gas CH_4. The composition of the biogas generated, varies to some extent depending on some factors such as; feedstock types, digestion systems, temperature, retention time, etc. Table 8 shows some average biogas composition values found in literature [192]. Considering biogas with a standard methane content of 50%, the resulting heating value is 21 MJ/Nm³, the density of 1,22 kg/Nm³ and the mass is similar to air (1.29 kg/Nm³). These gas produced are called biogas, can then be used as a chemical feedstock or bio-fuel. The AD process is quite similar to what naturally occurs in decomposing organic mud at the bottom of marshes or landfills. AD has been considerably developed and the technological advances in AD have recently increased the viability of this process. AD can help reducing pollution coming from agricultural and industrial operations. It is also one of the most efficient waste and wastewater

treatment processes, as it has been extensively used for the treatment of municipal sludge and a number of applications in the treatment of organic industrial wastes such as; fruit and vegetable processing wastes, packing house wastes, and agricultural wastes. Broadly, AD helps in reducing greenhouse gases, where an efficient AD system is designed to generate CH_4 with reduction in emission of greenhouse gas. The feedstock used in AD is sustainable, will not deplete as fossil fuels resources. AD also has a number of economical benefits as it converts residues into potentially saleable products, i.e. biogas. Moreover, AD contributes towards eliminating a wide range of pathogenic and fecal micro-organisms [192-194].

Table 8: Composition of biogas [192].

Compound	Chemical symbol	Content (Vol. %)
Methane	CH_4	50-75
Carbon dioxide	CO_2	25-45
Water vapour	H_2O	2 (20 °C) -7 (40 °C)
Oxygen	O_2	<2
Nitrogen	N_2	<2
Ammonia	NH_3	<1
Hydrogen	H_2	<1
Hydrogen sulphide	H_2S	<1

2.5.1 Historical Background Of AD

Anaerobic digestion is one of the oldest technologies in the field of production of biogas from biomass [195]. The AD process has evolved over the years. During the 10^{th} century BC, people in Assyria used biogas to heat bath water and in 16^{th} century the biogas was also used in Persia for the same goal. In the 17^{th} century, Jan Baptista Van Helmont stated that, flammable gases evolved from decaying organic matter. In 1776, Alessandro Volta depicted that there was an evidence of linkage between the amount of decaying organic matter and the amount of flammable gas produced. Three decades later or exactly in 1808, the production of methane by AD of cattle manure was demonstrated by Sir Humphry Davy.

Furthermore, the industrialisation of anaerobic digestion started specifically in 1859 with the first digestion plant in Bombay, India. By 1895, AD had appeared in England where biogas was recovered from a well-designed sewage treatment facility and fuelled street lights in Exeter city. Subsequent development of AD was due mainly to the development of microbiology. In 1930, anaerobic bacteria and the

conditions that enhance methane production were identified by research conducted by Buswell and others [196].

Prior to 1920, most AD processes were conducted in anaerobic ponds. The increase in understanding of the process led to greater control of conditions and to the use of closed tanks, and heating and mixing equipment to optimise AD. Due to coal and petroleum price reduction and their abundance, the production of methane suffered a setback then. During the Second World War, AD made a comeback as a result of the lack of fuel in Europe, but that did not continue for long. After the war AD suffered, a second setback, for the same reason as the first one.

AD was disregarded in some developed countries except as a wastewater sludge digestion technique, but some developing countries such as India and China embraced this technology. These countries saw a gradual improvement in small scale AD systems used mostly in generating energy and used for sanitation purposes. Also, the two energy crises in 1973 and 1979 caused a renewal of interest in the development of the AD process for the purpose of methane production as an energy source.

As its development is progressing, AD systems are becoming more complex and not limited to agriculture or animal waste treatment. AD is presently utilised in producing bio-products and pre-treating municipal waste. In previous years, two significant factors have caused Europe and others to further explore AD markets, which are; the high energy prices and strict environmental regulations. Today, many studies are conducting in a several countries to improve AD process for production of methane as a renewable energy source [195, 196].

2.5.2 General AD Process Description

Basically, the AD process is divided into four stages. These stages are as follows; 1) pre-treatment, 2) waste digestion, 3) gas recovery or gas upgrading, and 4) residue treatment. In order to obtain homogeneous feedstock, almost all digestion systems require pre-treatment of its substrates. The pre-treatment process 1) involves separation of non-digestible materials. The waste received by the digester is often source separately or mechanically sorted. The main aim of the separation process is to remove the un-necessary impurities i.e. stones, metals, etc. Also, all

recyclable materials such as; glass are removed from the organic waste. Mechanical pre-treatment such as grinding and beating can be used if source separation is not available. However, the resultant portion is then more contaminated leading to lower compost quality [195, 197]. The waste digestion stage 2) occurs in a digester and a variety types of digesters can be used with different temperatures, mixing devices, etc. The waste is separated before it is fed into the digester. Based on the solid contents of the feedstock, the digestion could be either wet or dry. Inside the digester, the feed is diluted to obtain the required solids content and is held in the digester for a specific time period. This time is known as retention time. The dilution can be done by using a wide range of water sources i.e. clean water, sewage sludge, or re-circulated liquid from the digester effluent. In addition, the temperature must be maintained at temperatures ranging from 30 °C – 65 °C, which can be done by employing heat exchangers. However, for receiving pipeline quality gas, 3) the biogas obtained in the AD must be scrubbed as it contains impurities. If the biogas produced is to be then used as natural gas or vehicle fuel, removal of carbon dioxide is crucially required. When a biogas is to be used for powering boilers and CHP, the removal of hydrogen sulphide and water are required. However, the last stage of AD process is the treatment of the residue. 4) The residues produced from AD commonly known as Digestate. It is a mixture of microbial biomass (generated by the digestion process) and undigested material in solid and liquid forms. Furthermore, the actual nutrient content of digestate is greatly dependent on the type of feedstock processed [195, 198]. According to Chambers [199], the following nutrient values are the typical nutrient values of digestate: Nitrogen (2.3- 4.2 kg/tonne), Phosphorous (0.2 - 1.5 kg/tonne) and Potassium (1.3 - 5.2 kg/tonne).

Moreover, during AD the volatile organic materials are not completely degraded as anaerobic microbes are able to convert only certain proportions of carbon into CH_4. The remaining carbon stays in the digestate in the form of Cellulose, Hemicellulose, Lignin, and protein. The quality of the digestate has a linear relationship with feedstock quality. Broadly, there are several treatment techniques can be applied on digestate prior utilising it in different applications such as, agriculture as fertiliser [195, 198, 200]. Recently, anaerobic digestion digestate has attracted attention in enhancing biogas yield, or in producing multiple bio-fuel

or bio-products. Lindner, J. et al. [201], investigated the optimisation of biogas yield from different feedstock by using mechanical pre-treatment only to the non-degraded digestate after the fermentation process for feeding it back into the process. Another objective of the study was to evaluate the losses of the volatile fatty acids (VFA) in the digestate, which may occur due to the heating of the substrate by the mechanical treatment, as well as its impact on the comminuting intensities and on degradation kinetics. In the study [201], a ball milling pre-treatment was applied to the digestate at four-time periods, which revealed that, mechanical pre-treatment resulted in a maximum to triplication of the methane production and to a quadruplication of the daily methane yield. Also, no losses of VFAs due to heating was observed.

2.5.3 The Biochemical Reactions In AD

Anaerobic digestion is a result of a series of chemical reactions among various groups of micro-organisms. During the chemical reactions, organic materials are decomposed through the metabolic pathways of naturally occurring micro-organisms in an oxygen-free environment. The decomposition process of biomass occurs in four stages: 1) hydrolysis/liquefaction, 2) acidogenesis, 3) acetogenesis and 4) methanogenesis. In the digestion process, the substrate must undergo all of these stages [197]. Figure 14 shows the decomposition process. However, each of these stages is described separately in this chapter.

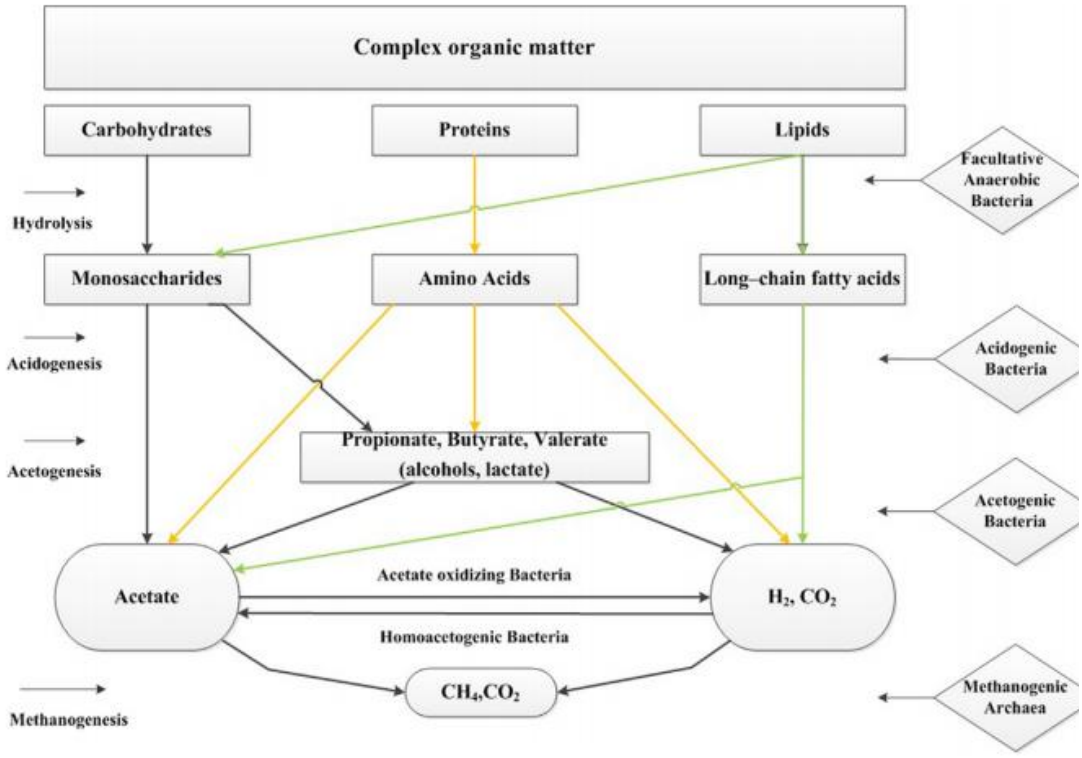


Figure 14: Decomposition stages of AD [202].

1) Hydrolysis

In theory, hydrolysis is the first stage of AD. As shown in Figure 14, in this stage, micro-organisms transform the particulate organic material into liquefied monomers and polymers such as; proteins, carbohydrates (i.e. polysaccharides, cellulose, lignin, starch and fibre) and fats [202]. These polymers are then converted to amino acids, mono-saccharides (i.e. sugar) and fatty acids respectively. The fermentative micro-organisms in which used to create monomers are then available to the next group of micro-organisms.

Raw cellulolytic waste as a feedstock are relatively slow at this stage as it is complex and contains lignin. Therefore, woody wastes are not preferable in anaerobic digestion process without pre-treatment. On the other hand, a polymer like carbohydrates are rapidly converted through this stage to simple sugars such as; glucose and subsequently fermented to volatile fatty acids (VFA) [202]. However, the rate of hydrolysis depends on different parameters i.e. pH, material composition, and particle size [203].

The following equation shows the hydrolysis reaction of a mixture of organic waste transformed into simple sugar; glucose [195, 202-204]:

$$C^6H^{10}O^4 + 2H_2O \rightarrow C^6H^{12}O^6 + 2H_2 \qquad \text{Eqn. 2}$$

2) Acidogenesis

In the acidogenesis stage, the acidogenic micro-organisms further breakdown the soluble organic molecules produced by the first stage into simple organic compounds, mostly; short chain volatile fatty acids, ketones, methanol, alcohols, hydrogen and carbon dioxide. The specific concentrations of the output products created in the acidogenesis stage, differ depending on the kind of micro-organisms, as well being influenced by some culture conditions, such as temperature and pH. The conversion of substrate from organic material to organic acid causes the pH of the system to decrease [195, 204-206].

3) Acetogenesis

In the acetogenesis stage, volatile fatty acids (VFA) and alcohols which are formed in Acidogenesis stage are oxidised into methanogenic substrates i.e. acetate, H_2 and CO_2. Both VFA and alcohols with carbon chains longer than two units and one unit respectively, are oxidised into acetate and hydrogen. Furthermore, the presence of hydrogen is necessary, as it plays a role of mediator and the reaction will only happen if the partial pressure of hydrogen is low enough to thermo-dynamically permit the conversion of all the acids. This reduction of hydrogen partial pressure is usually done by hydrogen scavenging bacteria. Thus, hydrogen concentration of a digester is a good indicator of the state of the AD process. In addition, acetogenesis and methanogenesis stages normally run parallel, as symbiosis of two groups of bacteria. Equation 3 below illustrates the transformation of propionate to acetate which is only achievable at low hydrogen pressure [195, 205, 207, 208]:

$$CH_3CH_2COOH^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \qquad \text{Eqn. 3}$$

4) Methanogenesis

This is a critical stage in AD process, as it is strictly influenced by operation conditions such as, composition of feedstock, feeding rate, temperature, and pH. In this stage, micro-organisms transform the hydrogen and acetic acid formed in the previous stage by the acid formers, into methane and carbon dioxide. The bacteria

used for the transformation in this stage are known as methanogens and they are strict anaerobes. Methanogens bacteria can produce methane either by cleavage of acetic acid molecules to generate methane and carbon dioxide, or by lowering of carbon dioxide by hydrogen. Usually, about two-thirds of the methane that produced in an anaerobic digester, resulted from the fermentation of acetic acid. While the remaining amount of methane is generated from the transformation of hydrogen and carbon dioxide by hydrogen-oxidizing methanogens. Equations 4,5 and 6 below show the reactions in this stage [38, 195, 208, 209]:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Eqn. 4}$$

$$2C_2H_5OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \qquad \text{Eqn. 5}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{Eqn. 6}$$

2.5.4 AD Stability Parameters

1. Temperature

As each microorganism species are active at different temperatures, temperature is considered as the most important factors influencing on the AD process and must be monitored and controlled carefully. Additionally, temperature has a major influence on the survival of pathogens during anaerobic digestion. In AD, there is a linear relationship between the rate of metabolic reaction and the temperature. [210, 211]

There are three main ranges of anaerobic digestion temperatures. The first one known as Psychrophilic range and ranges from 10 to 25°C. The second range is well known as Mesophilic range, approximately from 25°C to 45°C. Thermophilic is the third range, from approximately 45 to 70 °C. The optimum temperature of the Mesophilic and Thermophilic ranges are; 30 °C to 35 °C and 55 °C to 60 °C respectively. [197, 203]

Many studies have concluded that, lower temperature could lead to a decrease in the maximum specific growth and substrate utilisation rates. In the psychrophilic range, both chemical and biological reactions are progressed much slower than in mesophilic range. Psychrophilic temperature range was reported to affect biogas generation, methanogenic activity and microbial community composition as well.

Not only that, lower temperatures can lead to an exhaustion of cell energy, a leakage of intracellular substances or complete lysis. [212-214]

Thermophilic temperatures range has a number of advantages over the other two ranges. It has faster degradation rates, greater gas generation, less effluent viscosity and greater pathogen destruction [215]. On the other hand, thermophilic temperatures range has some flaws such as; high temperatures can lead to larger degree of imbalance [208].

However, the stability of temperature is important during digestion process, as the fluctuation of temperature can negatively affect the production of biogas. In comparison between mesophilic and thermophilic bacteria, mesophilic bacteria is less sensitive to temperature fluctuation of ± 3 °C, whereas, thermophilic bacteria is more sensitive to temperature fluctuation of ± 1 °C and need longer time to adapt to a new temperature [208]. It has been reported that, thermophilic temperatures can cause lower biogas production because of the production of volatile gases which restrains methanogenic activities. Based on Figure 15, it is obvious that there is direct relationship between the temperatures and the required retention time. Therefore, greater gas production is achieved if a digester operates in thermophilic conditions [207, 208]. Despite that, the majority of AD plants are carrying out the digestion process at mesophilic conditions (35-37 °C), as the process at this temperature is more stable and required lower initial energy expense [216].

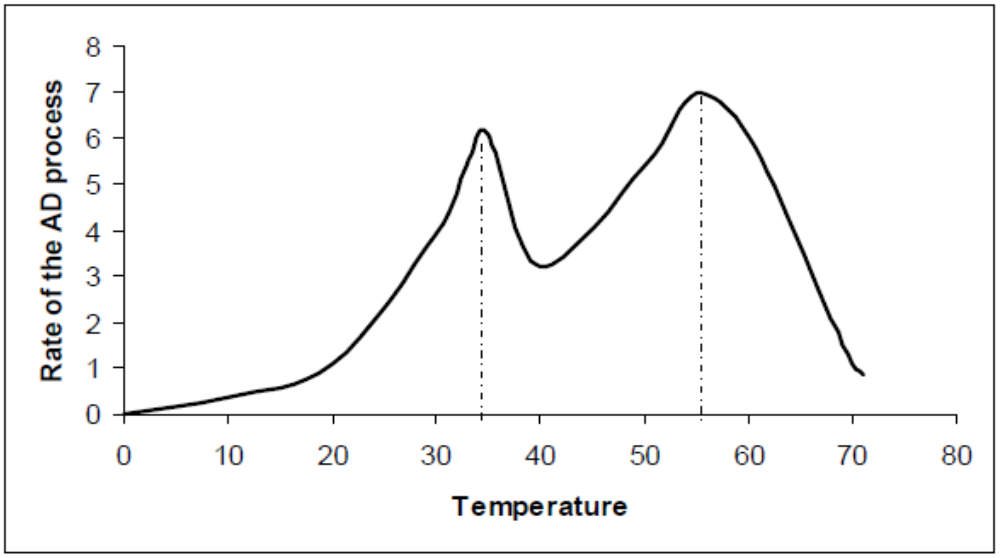


Figure 15: Temperature ranges in AD [207].

2. pH Level

pH value is the concentration of acid in aqueous systems, i.e. the concentration of hydrogen ions. pH level determines the equilibrium of the system and the stability of the digester. Within the digester, anaerobic bacteria, especially the methanogens bacteria, are sensitive to the acid concentration. The acidic situation slows down the growth of anaerobic bacteria. A pH level below 6 and above pH 8.3, can cause methane levels to fall off rapidly. [195]

Ward, et al. [216], reported that, the optimal pH of methanogenesis stage is about 7.0. While, the optimum pH of hydrolysis and acidogenesis stages is between 5.5 and 6.5 [217, 218]. As the optimal pH range for AD stages varies, therefore in order to achieve more efficient process, the application of two-phase reactors with a separate hydrolysis stage has been recommended by some designers. pH value is also affected by the retention time of residue and in a batch reactor acetogenesis occurs at a rapid pace. Additionally, an excessive particle size reduction of the substrate can speed up the hydrolysis and acidogenesis in the early stage of AD, resulting in accumulation of VFAs and decreasing pH level in the digester. Therefore in order to maintain the pH level at the optimal rate in the digester, all influencing factors should be taken into account. However, the reduction in pH level can be counteracted by the addition of lime or recycled filtrate achieved during residue treatment. Recycled filtrate can also eliminate the requirement of lime [195, 219].

3. Carbon To Nitrogen Ratio (C/N)

The relationship between the amount of carbon and nitrogen in the organic materials is expressed by the C/N ratio. The optimum C/N ratio of anaerobic digesters can be obtained by mixing high and low C/N materials, e.g. organic solid waste mixed with animal manure. The optimum C/N ratios range from 20 to 30 [195]. A low C/N ratio results in an accumulation of ammonia and an increasing in the pH value to more than 8.5, which is toxic to methanogenic bacteria. On the other hand, a high C/N ratio indicates a rapid consumption of nitrogen by methanogens which leads to a lower production of gas [195].

4. Mixing

The benefit of mixing inside a digester, is to blend fresh materials with residues containing microbes. This enhances contact between micro-organisms and the

substrate. Mixing prevents the formation of scum and avoids a drop of the temperature within the digester. Mixing process normally distributes heat and bacteria evenly within the digester. Slow mixing is preferred rather than excessive mixing as the excessive mixing can disrupt the microbes, thus resulting in a reduction in the production of biogas [195, 216]. Slow mixing enables the digester to better absorb the disturbance of shock loading compared to excessive mixing conditions [220]. Stroot, P.G. et al. [221], stated that, continuous mixing is not necessary for efficient performance and is inhibitory at higher loading rates. Moreover, the reduction of mixing levels can be implemented as an operational tool to stabilise unstable digesters. The type of mixing equipment and amount of mixing varies with the type of reactor used, and the solids used in the digester [195].

5. Moisture Content

Moisture content simply is the proportion of water in the biomass. Equation 7 is used to calculate moisture content of the biomass [222]:

$$\text{Moisture } \% = 1 - \frac{M_c - C}{M_f - C} \times 100 \qquad \text{Eqn. 7}$$

Where: M_c: the mass of dry matter and container [g], C: the mass of the container [g], and M_f: the mass of fresh sample and container [g].

However, moisture content is one of most important factors influencing an AD process. It has to be controlled and maintained carefully in the process, as if it is too high, then the readily degradable organic matter may dissolve and therefore the process would be negatively affected. As the digestion process is classified as wet and dry according to the quantity of TS in the feedstock, TS of wet digesters ranges from 16% and below, whereas from 22 to 40% for dry reactors. Digesters with value of TS falling between the TS of wet and dry digesters (17 to 21%) are called ‘semi dry’ [207]. The dry reactor technology is commonly used with municipal solid waste (MSW) or vegetable wastes rather than with manures. While, wet reactor is used with many types of manure. In addition, many studies have proved that, the greatest methane yield rates can be reached at 60-80% of humidity [216, 222, 223]. Previous results have revealed that, specific methanogenic activity at low percentage of moisture content is significantly lower than that at high moisture

content. Particularly, as the specific methanogenic activity can increase linearly when the moisture content of the feedstock's is increased from 65% to 82% [224].

6. Toxic Substances

The existence of toxic inhibitory compounds can negatively influence digestion process. There are many organic and inorganic toxics as well as inhibitory substances which can inhibit or affect an anaerobic digester. Ammonia, sulfide, sight metal ions, heavy metals, pesticide residues, sheep dip, hydrogen sulphide H_2S etc. are some of the well-known toxic substances in anaerobic digesters [225]. Thus, toxic substances must be kept at a minimum level in the feedstock.

2.5.5 AD Operational Parameters

In AD, the rate at which the micro-organisms grow is crucial. Among the different operational factors of the digester that influence the AD process, the most important factors must be controlled and monitored to enhance the microbial activity and therefore increase the efficiency of the anaerobic degradation process. Some of the important factors are discussed below**:**

1. Retention Time

The retention time or the residence time is the time required to obtain complete degradation of the organic matter. Inside the digester, the feed is diluted to achieve the required solids concentration and also that it remains in the digester for a specific time period. This time is known as retention time. However, the required retention time for completion of the AD reactions varies with differing technologies, process temperature, and waste composition. In other words, retention time is the average time it takes for organic material to digest. The longer a substrate is kept under proper reaction conditions, the more complete its degradation will be. The retention time for materials processed in mesophilic digester ranges from 10 to 40 days. But in the thermophilic digester range, lesser retention times of around 12-14 days are required [195]. For example, a high solids reactor performing in the thermophilic range has a retention time of 14 days. Furthermore, decreasing retention time, reduces the size of the digester leads to cost savings. Thus, there is a need to design a system that can obtain a complete digestion in shorter residence time. A shorter residence time results in greater production rate per reactor volume

unit, but a lower overall degradation. These two effects must be carefully maintained in case of designing full-scale reactor [195, 204, 226].

2. Waste Composition/Volatile Solids (VS)

Usually, the wastes treated in an AD comprise of, bio-degradable organic materials, combustible materials, and inert materials. Firstly, bio-degradable organic materials include food residue, grass and tree cuttings. Secondly, the combustible materials include slowly degrading lignocellulosic organic matter that contain coarser wood, and paper. The lignocellulosic organic matter is difficult to degrade in an anaerobic situation and is better suited for waste-to-energy plants. Lastly, inert materials include stones, glass, sand, metal, etc. must be removed and recycled or sent to landfill. As these materials could increase digester volume and wear of equipment, the removal of these materials before digestion is crucial. The composition of waste can greatly affect the quality of biogas yield as well as resulting compost quality.

The volatile solids (VS) is the dry matter, which is evaporated by combustion at 575°C. It is measured by subtracting total solids (TS) from the ash content as achieved by complete combustion of the feed wastes. VS normally consists of a bio-degradable volatile solids (BVS) portion as well as a refractory volatile solids (RVS) portion. Waste characterised by high volatile solids (VS) and low refractory volatile solids (non-biodegradable materials), is best suited for AD treatment [195]. Moreover, VS is very important parameter. It must be controlled carefully where, the excessive feedstock concentration in the digester could result in imbalances of the bacterial population, VFA accumulation and digester failure. While, the extremely low substrate concentration can lead to starving condition inside the digester and therefore a reduction in the methane generation [227-229].

3. Total Solids Content (TS)/ Organic Loading Rate (OLR)

TS refers to the total solids content. It is simply the dry matter of a sample in which is heated at 105 °C until a constant weight was achieved. TS has an inverse relationship with the reactor volume. As the TS increases, the reactor volume decreases [195]. Low solids (LS) of AD systems contain less than 10% TS, medium solids (MS) about 15-20% and high solids (HS) about 22% to 40% [230].

OLR is referred to the Organic Loading Rate. OLR is the amount of VS placed into the digester, or in other words, the quantity of dry organic matter fed per unit volume of the digester per unit time. Equation 8 represents that;

$$OLR = \frac{m * c}{VR} \quad \text{Eqn. 8}$$

In this equation, OLR represents the organic load in (kg/days.m^3). Where, m is the mass of substrate fed per time unit (kg/days), c is the concentration of organic matter (%), and VR is the digester volume (m^3). However, feeding the system more than its maximum OLR, could negatively results in low biogas yield because of the accumulation of inhibiting materials such as fatty acids in the digester slurry. If this happens, the feeding rate to the system must be reduced [195]. However, OLR is an important controlled factor especially in continuous systems. Many plants have reported system failures due to overloading [197].

2.5.6 Feedstocks

There are many different types of feedstock that can be used in AD including cattle and pigs manure, slurry, vegetables processing residues (e.g. sugar beet), dairy processing residues (e.g. cheese processing). Recently, several energy crops (grains, maize, grass silage), have been widely used as feedstock in purpose of biogas production in some countries such as; Austria and Germany. As the composition of feedstock can greatly affect the nature of emission and output of gas, it requires careful assessment. Furthermore, toxic substances must be kept at a minimum level in the feedstock as they inhibit the digestion. Some of these toxic substances are pesticide residues and sheep dip. There are also specific materials that must never be added to a digester as they limit or kill the process. In addition, long straw and non-biodegradable materials can cause blockages in the system and must be avoided. The quantity and quality of feedstock for a digester should be considered as they can maximise the quantity and quality of the outputs. Thus, there is an economic and environmental benefit from using feedstock. [225, 231-233]

In order to produce methane from organic substrates, there are two primary factors should be taken into account, which are; the composition and the biodegradability of the substrates. Cellulose, hemi-cellulose, starch, sugar and the crude substances of protein, fat, and fibre have high influence on the methane

production [234]. Plant usually captures energy in its cell wall as starch. Plant cell wall contains polysaccharide that consists of long chain of simple sugar; glucose produced by the plants during photosynthesis process. High concentrations of glucose found in plants can be transformed to produce energy. Moreover, lignocellulosic biomass as an energy source, is the most abundant materials in the world for the production of bio-fuels. It is mainly composed of carbohydrate polymers such as, cellulose, hemicellulose and lignin [20]. These polymers are briefly described below:

1. Lignocellulosic Biomass

Lignocellulosic biomass is widely available everywhere in low and at stable costs. It is mainly waste materials that are containing in abundance of carbohydrates. In addition to that, it is non–competitive as a food chain [20, 21].

Lignocellulosic biomass sources are classified into number of groups, they are the following; 1) energy crops (perennial grasses and other dedicated energy crops), 2) aquatic plants (water hyacinth), 3) forest biomass and wastes (softwood and hard wood, sawdust, pruning and bark thinning residues), 4) agricultural residues (cereal straws, stovers and bagasse), and 5) organic fraction of municipal solid wastes (MSW) [235, 236]. Figure 16 below shows lignocellulosic biomass sources groups and examples for each group**.**

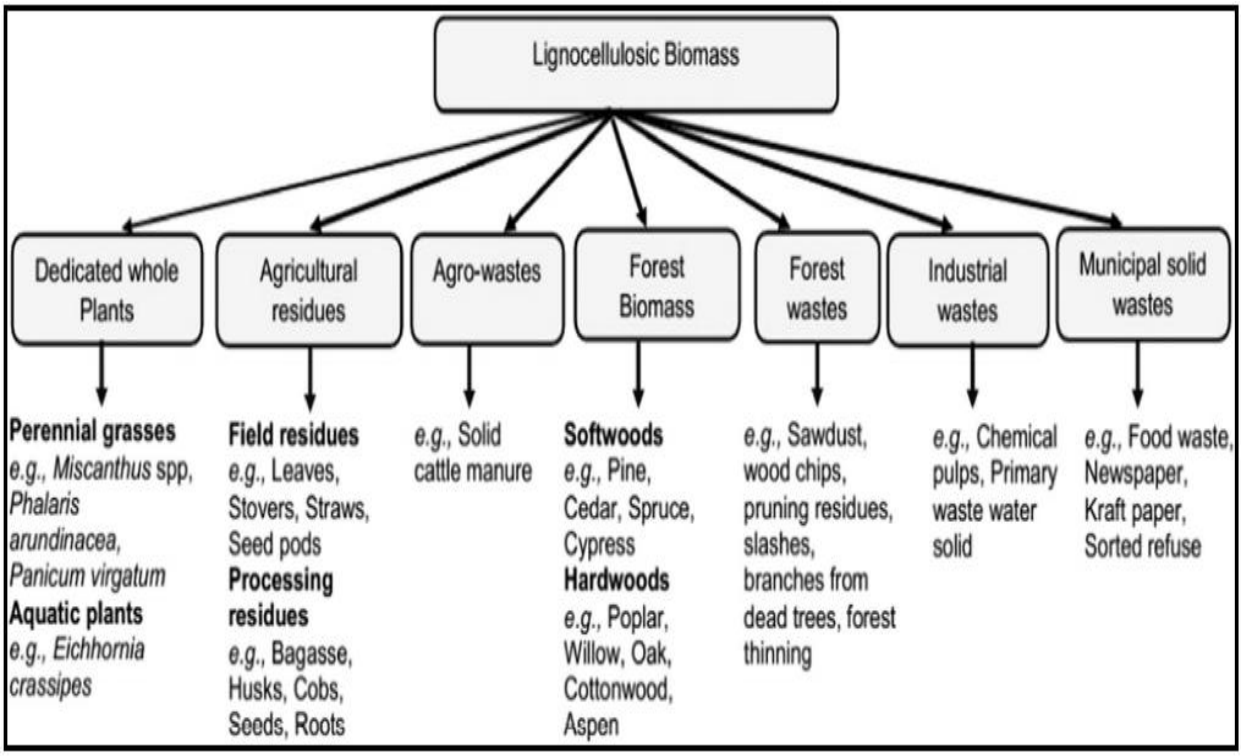


Figure 16: Lignocellulos biomass groups and examples [20].

The main constituents of lignocellulosic materials are cellulose, hemicellulose, and lignin, that is polymers that are closely linked with each other constituting the cellular complex of the vegetal biomass [237]. Cellulosic and hemicellulosic portions of biomass are transformed to mono-saccharides which can be subsequently fermented to biogas. There are some properties of lignocelluloses cause slowing or inhibiting to the biodegradation process of untreated lignocelluloses i.e. lignin content and cellulose crystallinity. It results in extent of degradation not more than about 20%. Therefore, in order to obtain optimal biogas yield, strong pre-treatment process are required for this type of biomass [238].

- ***Cellulose***

Cellulose is an essential organic compound (polymer) of lignocellulosic biomass (35– 48%) [239]. It has high molecular weight linear homopolymer of repeated units of cellobiose [237]. It is consisting of long chains of glucose monomers linked to each other by Glycosidic bond. These bonds cause the cellulose to be filled into microfibrils and give it unique properties of both mechanical strength and chemical stability. Cellulose micro-fibrils compose of two crystalline forms. These micro-fibrils have high crystalline (around two third) and less amorphous forms. Crystalline cellulose normally consists of high fraction of

cellulose, whereas a small fraction of unorganised cellulose chains forms amorphous cellulose. Cellulose is highly susceptible to enzymatic degradation in its amorphous form. However, pre-treatment of lignocellulosic biomass before enzymatic hydrolysis is an important step [240, 241].

- ***Hemicellulose***

Hemicellulose is a major polymer compound in lignocellulosic biomass and it makes up of about (22–30%) of it [239]. Hemicellulose is composed of five different sugars, which are; L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose, and other components i.e. acetic, glucuronic, and ferulic acids [237]. Hemicellulose is attributed to a group of heterogeneous polysaccharides which are created through biosynthetic routes different from that of cellulose. As with cellulose, hemicellulose function is supporting material in the cell wall. Hemicelluloses structures are weak and easier to be hydrolyzed than cellulose. In comparison with cellulose, hemicelluloses are mixed polymers not pure like cellulose, they have low molecular weight polymers, while cellulose has high extent of polymerisation and hemicellulose are higher than cellulose in term of solubility and susceptibility to hydrolysis. The majority of hemicelluloses have an extent of polymerisation of only around 200 degree [242].

- ***Lignin***

Lignin is the third major organic polymer compound of lignocellulosic (15-27%) [239]. The amounts of lignin differ from one plant species to another. Lignin is very abundant and comes after cellulose in terms of availability. However, lignin is a three-dimensional amorphous polymer and has a complex molecule structure which differs based on the biomass sources as well as the extraction application used. Lignin tightly holds the other two carbohydrates polymers together. Its role is to provide rigidity and cohesion to the material cell wall. It also provides water impermeability to the xylem vessels and creates a physical and chemical barrier against microbial attack oxidative stress. Furthermore, lignin is very resistant to chemical and enzymatic degradation because of its molecular structure. Due to that, lignocellulosic materials are used in anaerobic digestion process. Whereas, the purpose of the pre-treatment is to modify the properties of lignocellulosic materials for better exposure to chemical and biological degradation [237, 240, 243].

2.5.7 The Challenges Of AD

Despite all of the advantages AD can offer such as; its contribution in reducing greenhouse gases and converting residues or no-valued products into high-value bio-based products, there are number of issues that must be considered especially when it is applied extensively. As already stated, the failure in solving these issues may result in reducing the effectiveness and sustainability of AD, limiting the investment in it and making it less attractive. However, these issues are classified as operational, environmental and economical issues. They are described below in more details below:

1. Operational Issues

Multiple factors can negatively affect the performance of AD and result in poor operational stability of the system, such as; inadequate operational management, lack of process control and external disturbances. In order to ensure efficient organic waste stabilisation and constant, prevent system failure, stable biogas production and therefore maximise process efficiency, these factors must be taken into account in advance. In addition to that, the personnel operating the system must be qualified enough to ideally monitor and control the system. [54, 55].

2. Environmental Issues

The accumulation of large quantity of AD digestate and the improper handling of it could cause serious environment issues. For limiting or stopping such issues, many studies have assessed the possibility of taking advantages of the digestate in several aspects, such as [25, 200, 201, 244, 245]:

- Utilising it as AD by-products. AD digestate are used as whole digestate or can be separated into two products. After separation of digestate by commercial screw press separator or others, it can be divided into two streams rich in nutrients, such as: (a) nutrients-rich liquid digestate, and (b) nutrients-rich solid digestate. Liquid digestate is commonly used in agricultural as a bio-fertiliser. The amount applied to the land must be carefully regulated, as the excess application of digestate would cause an environmental issue. Both the liquid digestate and whole digestate are good sources of readily available nitrogen. While, fibre or as is well known solid digestate looks like compost. It is a good source of stable organic matter.

- ❖ In order to increase the biogas yield, the digestate is re-digested again.
- ❖ After pre-treating of the solid digestate, it can be used as substrate in producing bio-fuels (i.e. bio-diesel and bio-ethanol).

3. Economical Issues

The fluctuation of fossil fuel price indexes, the amount of energy required to produce the biogas by AD and others are very important factors and must be considered prior investing in AD or applying it at large scale. Previous study on the production of biogas from Irish algae, calculated the energy balance related to the use of the mechanical pre-treatment. The energy balance was carried out by comparing the energy content of the biogas produced by the raw biomass against the energy content of the biogas produced by using mechanically (beating) pre-treated biomass. The energy consumed in the pre-treatment was the only energy considered in the study. In order to evaluate the economic viability of the use of seaweed biomass for biogas production at a larger scale, the study suggested measuring the energy consumed in the digestion process as well. [3, 27, 33].

In general, solving of the above issues radically would greatly help to increase the investment and the dependence on the anaerobic digestion to convert biomass into high-value bio-products. However, finding these radical solutions requires further study.

2.5.8 Achieving Of Development And Prosperity In AD Plants Worldwide

There are many AD plants in many European countries. Germany plays leading role in this field. The number of AD plants in Germany has remarkable increased from 4136 in 2010 to nearly 8900 plants in 2015 [246, 247]. The Republic of Ireland is an emerging European country in the this field. According to the Irish Ministry for Agriculture, Food and the Marine, the number of AD plants in Ireland was 31 plants in 2014. Compared to 8900 plants in Germany and 1900 in Italy in 2015 therefore, the number of the AD plants in Ireland is relatively small. Furthermore, the government of the Republic of Ireland is under pressure to reduce carbon emissions through the exploitation of the agricultural waste in producing bio-energy [248]. The report of the Sustainable Energy Authority of Ireland in 2017 revealed the potential of producing biogas from slurry and silage in Ireland. These

biogas supplies would contribute, (according to the projections) to 28% of the total gas supply in 2025 and to approximately 50% in 2050 [249].

Due to the negative impacts of fossil fuel and its derivatives on the humans' health and others, the shift to renewable energies has become a requirement not an alternative [250]. Therefore, many countries have reduced their use of non-renewable energy and substitute them with renewable energy. The Kingdom of Saudi Arabia is one of the largest producer country of oil and has the second-largest reserves of naturally occurring oil in the world. The revenues of the Kingdom from oil, represent the largest revenue compared to its other revenues [251]. In order to diversify the sources of income and because of the consequences of using fossil fuels, decreasing the usages of the non-renewable energy and increasing of the production and the dependence on the renewable energy are some of the major pillars of the Kingdom's vision towards 2030 which was released in 2015 [252]. According to the 2020 budget report for the Kingdom of Saudi Arabia [253], the public revenue has increased from 520 SAR billion in 2016 to 833 SAR billion in 2019 and, expected to reach 833 SAR billion in 2020. The non-oil revenues of the Kingdom have increased by 148% since 2015. It is also expected to increase more in 2020 by 1.6%. On the other hand, it is expected that the Kingdom's oil revenues will continue to decline in 2020 by up to 14.8% of oil revenues in 2019 [253].

The number of sewage treatment plants in the Kingdom of Saudi Arabia are getting larger. They are now available almost all over the Kingdom. The treated sewage water is utilised in industries and for irrigation. So far, the generated sludge is not exploited in producing biogas and only used in agricultural applications as a soil improver or bio-fertiliser [254]. Additionally, AD is one of the common processes in many countries that has proved its significant potential in producing biogas [181, 255]. Despite that, it is still not yet applicable in many countries such as; Saudi Arabia.

However, increasing the dependence on AD biogas either in the emerging countries or in the countries which aimed to increase its production on renewable energy, requires investigating the major common issues and obstacles facing AD plants. This would significantly help those countries solving or avoiding the issues in advance and making the investment in AD more desirable.

2.6 Biomass Pre-Treatment

Pre-treatment is essentially used to improve the bio-digestibility of the biomass, to increase accessibility of the enzymes to the biomass, and to improve the rate of hydrolysis of material being fed into digesters in order to increase the total methane yield [30]. Moreover, the best effect of the pre-treatments is mainly dependent on the composition of the biomass and operating conditions [256]. In general, many pre-treatment methods have been tested in several studies on various substrates. These pre-treatment methods can be categorised into the following; physical (mechanical) such as; milling, beating, ultrasonic and collision plate pre-treatment, chemical (e.g. alkaline and acid pre-treatment), and biological (e.g. fungal pre-treatment). According to Carsson, et al. [257], pre 2012 the majority of biomass studied using pre-treatment studies were the residues of waste water treatment plant (WWTP) and mostly using thermal and ultrasonic (mechanical) pre-treatment methods. Energy crops/harvesting residues, organic fraction of municipal solid waste (OFMSW), organic waste from food industry and manure respectively, are the more widely biomass have been investigated after WWTP residues. In addition, chemical and thermal pre-treatments were the typical pre-treatments methods utilised on energy crops/harvesting residues, organic waste from food industry and manure. Whilst, mechanical and thermal pre-treatments were mainly applied to OFMSW. Lignocellulosic materials are very complex, thus pre-treatment is difficult. Hendriks, and Zeeman, [258], concluded that, there are number of factors negatively influence the digestibility of hemicellulose and cellulose, which exist in lignocellulosic biomass. Some of the main factors are; the crystallinity of cellulose, particle size, lignin and moisture content, available surface area and the extent of cellulose polymerisation and the acetylation of hemicelluloses [258, 259]. Moreover, the effect of pre-treatment methods on the chemical concentration and physical properties of biomass varies from one biomass to another. Table 9 depicts the influence of some pre-treatment methods on the compositional and structural alteration of lignocellulosic materials [260]. The next few sections describe three broad pre-treatment methods; mechanical, chemical and biological pre-treatments.

Table 9: The effects of different pre-treatments on compositional and structural alteration of lignocellulosic materials (Adapted from [258]).

Pre-treatment	Increase of access-ible surface area	De-crystallization of cellulose	Solubilization of hemicelluloses	Solubilizat-ion of lignin	Alter-ation of lignin structure	Formation of furfural/ hydro-xymethlfurfu-ral (HMF)
Mechanical	√	√				
Irradiation	√	×	×			×
Steam-explosion	√		√	×	√	√
Liquid hot water	√	ND	√	×	×	×
Catalyzed steam-explosion	√		√	√/×	√/×	√
Acid	√		√	×	√	√
Alkaline	√		×	√/×	√	×
Oxidative	√	ND		√/×	√	×
Ionic liquid	√	√	×			
Thermal acid	√	ND	√			√
Thermal alkaline	√	ND	×	√/×	√	×
Thermal oxidative	√	ND	×	√/×	√	×
Ammonia fibre explosion	√	√	×	√	√	×
Biological pre-treatment	√	ND	√	√	√	

√= Major effect, ×= Minor effect, ND= not determined, and blank= no effect

2.6.1 Mechanical Pre-Treatment

The main objective of mechanical pre-treatment is to reduce particle size and the crystallinity of the substrate. The reduction in particle size results in an increasing of available specific surface as well as a reduction of the degree of polymerisation. The increase in available specific surface and the reduction in the degree of polymerisation greatly contribute to the increase in the rate of hydrolysis. In comparison to chemical and thermo-chemical pre-treatments, mechanical pre-treatment has a major advantages in that, no toxic or inhibitory substances are generated during the disintegration step [258]. Presently, several mechanical pre-treatment methods are commonly applied in the pre-treatment of many types of biomass. These methods vary in their effectiveness in the pre-treatment of biomass and lead to different results [260]. Table 10 shows the biogas yield from different mechanical pre-treatment methods of feedstocks at certain pre-treatment conditions. As a results and in order to investigate the optimal economical and environmental

pre-treatment method, more comparisons between pre-treatment of various biomass methods are required.

Table 10: Biogas yield from different mechanical pre-treatment methods, Adapted from [260].

Physical pre-treatment	Feedstock	Pre-treatment conditions	Results
Comminution	Agricultural residuals: wheat straw, rice straw, oat, clover, bagasse, coconut fibre, hemp, banana peelings, cauliflower leaves, and digested biofibres. Forest residuals: mirabilis leaves Grass: dump grass and grass hay Municipal solid waste (MSW): organic fraction of MSW (OFMSW)	Particle size: 0.003-30 mm	Up to 30% improvement of methane yield. Occasionally, reduced size decreased biogas production.
Steam-explosion	Agricultural residuals: wheat straw, corn stalks, corn straw, citrus waste, potato pulp, rape straw, and digested bio-fibres Hardwood: Japanese cedar, willow, and birch Grass: bulrush, Miscanthus, and seaweed MSW: OFMSW and paper tube residuals Softwood: bamboo	160-260 ^{0}C, 0.7-4.8 MPa, and seconds to a few minutes	Positive effect of up to 40% increase in biogas yield. Occasional zero or negative effect also occurred.
Liquid hot water (LHW)	Agricultural residuals: Wheat straw, rice straw, oil palm empty fruit bunches (OPEFB), sunflower stalks, and sugarcane bagasse Grass: Miscanthus and	100-230 ^{0}C (0.1-2.8 MPa) for a few minutes to hours	7-220% increase of methane yield

	hybrid grass MSW: OFMSW and paper tube residuals		
Extrusion	Agricultural residuals: barley straw, maize, and solid fraction of manure Grass: Lolium multiflorum and pelleted hay MSW: OFMSW	0.45-3.5 MPa for a few minutes (e.g. 4-12 min) and typical temperature of 60-90 ^{0}C	8-70% improvement of methane yield
Irradiation	Agricultural residuals: wheat straw, barley straw, spring wheat, winter wheat, oat straw, and rice stalks Grass: switch-grass and hybrid grass MSW: OFMSW	115-300 ^{0}C for a few minutes to hours	4-28% improvement of methane yield. No or adverse effects were found. Combination with acids or alkali could achieve greater improvement of biogas yield.

1. <u>Beating Pre-Treatment</u>

Beating pre-treatment is one of the most recent mechanical pre-treatment techniques used. It was first introduced by the bio-energy group team in the School of Mechanical and Manufacturing Engineering in Dublin City University (DCU) based on employing a Hollander Beater device [261]. It is not widely applied yet in the pre-treatment of biomass. The Hollander Beater was used to treat biomass like; seaweeds and lignocellulosic materials, resulting in the disruption of the crystallinity of the substrate and reduction in particle size, thus improved hydrolysis rate and subsequently methane yield [32, 33]. The Hollander Beater model "Reina", is used in this study for pre-treating of lingocellulosic biomass. This device was developed during the period 1660 and 1682 by Dutch scientists. At that time, the main function of this device was to produce paper pulp from cellulose containing plant fibres [262].

A number of studies have been carried out using this pre-treatment method of different biomass prior to AD for biogas production. Recently, a study [33]

compared three different mechanical pre-treatment techniques; microwave, milling and beating techniques of seaweed as a biomass. The study discovered that, the greatest performance in terms of methane production was obtained when the beating pre-treatment was applied, whereas microwave and milling techniques negatively affected the digestion process. Aboderheeba, A. [32] also employed the beating technique for pre-treatment of maize silage, fresh grass and potato waste as biomass and concluded that, the beating treatment was as a new pre-treatment technique effective and accelerated the degradability for lignocellulosic material.

2.6.2 Chemical Pre-Treatment

Chemical pre-treatment aims essentially to destruct organic compounds by means of strong acids, alkalis or oxidants [263]. It includes various chemical pre-treatment methods, such as; alkali and acidic pre-treatment. The effectiveness of chemical pre-treatment methods varies depending on the type of method used and the characteristics of the feedstock. Therefore, the selection of the best pre-treatment method is a crucial step. Moreover, applying of chemical pre-treatment on those materials rich in carbohydrates and easily bio-degradable, is not recommended as it would result in failure of the methanogenesis step [264]. Whereas, applying it on materials rich in lignin can have a positive impact [265].

2.6.3 Biological Pre-Treatment

Biological pre-treatment includes aerobic and anaerobic methods. It can also include the addition of specific enzymes such as peptidase to an AD system [266]. In general, this type of pre-treatment employs microorganisms, predominantly fungi, which can generate enzymes which have a potential to degrade recalcitrant compounds like, lignin and hemicellulose, since cellulose has higher resistance to biological attack. As with any pre-treatment method, biological pre-treatment has advantages and disadvantages. The most important features that characterise biological pre-treatment are; it does not require high power, the elimination of any chemical requirements, and it is performed under mild environmental conditions [31]. Whilst, the most common disadvantages which can reduce its significance are; the length of retention time in days, it requires careful growth conditions and large space is needed to carry out biological pre-treatments [267]. Basically, the primary objective of biological pre-treatment is to decrease the loss of carbohydrates and increase the lignin elimination for AD substrates with high digestibility [260].

2.7 Biogas

AD biogas is a valuable potential energy and suitable replacement of fossil fuel. The production of biogas has widely increased in many countries [268]. In Europe, Germany was first in the production of biogas [269]. Where, the numbers of biogas plants have rose from 139 up to 8726 in the period of 1992-2014 [270] and reached approximately 8900 plants in 2015 [246, 247]. In the recent years, the tendency to use biogas has increased in many countries. There are numerous types of biogas plants in Europe, classified based on the type of substrates and the technology applied or their size. Presently, biogas technology is still under evolution in some countries, like Greece and Ireland, as well as in many of the new, Eastern European, member states, where a large biomass potential is identified [271]. The next few sections discussed some different aspects of biogas produced through AD.

2.7.1 Biogas Composition

Biogas produced through AD is mainly composed of a number of gases in different proportions. Typically, the composition of biogas produced from AD depends on the feedstock, the organic matter load and the feeding rate of the digester. Generally, the mixed gas is saturated with water vapour and may be dust particles and siloxanes as well. The composition of biogas is normally similar to a large extent, to landfill gas composition but different from the natural gas. Landfill is composed of large amounts of halogenated compounds. It can contain high amounts of oxygen if too much air has been drawn in during collection process [38, 39]. The calorific value of typical biogas (60 % CH_4 and 40 % CO_2) ranges from 5.5 to 6.5 kWh/m^3, this is promising compared to natural gas, which has an energy value of 5.8-7.8 kWh/m^3 [272]. Thus, biogas has the potential to be a significant replacement for natural gas. Table 11 below shows the typical composition of biogas, natural gas and landfill gas with the amount of each constituent.

Table 11: Biogas, natural gas and landfill composition [38].

Constituents	Units	Natural gas	Biogas	Landfill gas
Methane CH_4	Vol. %	91	55-70	45-58
Ethane C_2H_6	Vol. %	5.1	0	0
Propane C_3H_8	Vol. %	1.8	0	0
Butane C_4H_{10}	Vol. %	0.9	0	0
Pentane	Vol. %	0.3	0	0
CO_2	Vol. %	0.61	30-45	32-45
Nitrogen N_2	Vol. %	0.32	0-2	0-3
Volatile organic compounds (VOC)	Vol. %	0	0	0.25-0.50
Hydrogen H_2	Vol. %	0	0	Trace to < 1%
Hydrogen sulphide H_2S	Ppm	~ 1	~ 500	10-200
Ammonia NH_3	Ppm	0	~ 100	0
Carbon dioxide CO	Ppm	0	0	Trace

2.7.2 Biogas Uses

Biogas can be used instead of natural gas in all natural gas appliances. Gas properties used in gas appliances differ from each other. There are major variations between the requirements of stationary biogas applications and fuel gas or pipeline quality. Therefore, upgrading biogas technology may be further required to meet an appliance standard [38, 273]. Biogas can be used in several fields such as; heating, CHP, fuel cells and fuel for vehicles.

2.7.3 Gas Upgrading Technologies

The biogas produced from the digestion stage, within an AD process generally contains low amounts of various impurities such as; hydrogen sulphide, ammonia, oxygen and nitrogen. In order to use the biogas further in several fields, upgrading is required. The primary reason of that, is to avoid corrosion and mechanical wear of appliances where biogas was used [40]. As stated before, H_2S causes corrosion to an appliance, thus, the removal of H_2S before most upgrading systems is important. Removal of H_2S can be done by an addition of iron hydroxide to the digester. In the case of the biogas which contains large amounts of H_2S (i.e. over 2000 ppm), the use of a H_2S bio-scrubber would be vital before the removal of CO_2 [274, 275]. After biogas is upgraded, it can be injected into either the gas grid or used as a transportation fuel in compressed natural gas (CNG) motor vehicles. The final product is in practical quite similar to natural gas and can be mixed as bio-natural gas or sold separately [276]. However, there are number of technologies available for scrubbing contaminants and upgrading gas to the required gas quality. Some of

these technologies are outlined below. Not all of these technologies are preferable for the application with biogas in regard to the cost and/or environmental aspects.

1. Carbon Dioxide Removal

The removal of CO_2 significantly helps in improving the energy of the gas to meet vehicle fuel standard or may be natural quality standard. Four different methods are currently available in market and can be utilised for such a purpose, which are [38]:

- Water scrubber
- Polyethylene glycol scrubbing
- Carbon molecular sieves
- Membranes separation

2. Hydrogen Sulphide Removal

To avoid corrosion and mechanical wear of an appliance in which the gas is used, H_2S must be removed. The following methods are widely used to do that [38]:

- Air/oxygen dosing to digester biogas,
- Iron chloride dosing to digester slurry.
- Iron hydroxide or oxide.
- Iron oxide pellets.
- Activated carbon.
- Water scrubbing.
- NaOH scrubbing

3. Halogenated Hydrocarbon Removal

It can be removed by either using of pressurised tube exchangers filled with specific activated carbon or the same methods used to remove CO_2. Halogenated hydrocarbon causes formation of corrosion in CHP engines, valves, spark plugs, etc. Therefore, the producers of CHP engine claim maximum allowed limits of halogenated hydrocarbons in biogas. [34, 265, 269]

4. Siloxanes Removal

Organic silicon compounds are sometimes existing in biogas from landfills and from sewage sludge. In the separation of the compounds from biogas, Activated carbon methods may be applied. It is very efficient method but very costly.

Absorption in a liquid mixture of hydrocarbons method can be used in order to remove organic silicon compounds from the biogas. In addition, reducing of the amount of siloxanes present in biogas can be obtained through cooling the gas and separating the condensed liquid. [38, 277]

2.7.4 Production Of Biogas From Fruits And Vegetable Wastes As Lignocellulosic Materials

There are many several studies have been carried out on the biogas production from different types of fruits and vegetables as biomass. Jaafar, K. A. [278], carried out a study on the verification of the possibility of producing biogas from Iraqi Zahdi date palm waste as a fruit biomass, with waste water treatment activated sludge in thermophilic AD. Another objective of the study was to evaluate the impacts of extra nutrients addition to the digestion mixture. At that time this study was the first study in investigating biogas production from Iraqi date palm, where, the study produced 67% methane from total volume of biogas produced and 0.57 litres for each gram volatile solid of substrate within a short time cycle of biogas production. The production in short time cycle can be considered as very significant and promising results for Iraqi date palm in the production of biogas.

Sagagi, et al. [279], implemented a study on the production of biogas from five different types of fruits and vegetables wastes. The study weekly evaluated the output biogas from pre-treated wastes of cow dung (control), pineapple, orange, pumpkin and spinach. The average volume of weekly biogas produced from each substrate were; 1554, 965, 612, 373 and 269 cm^3 respectively. The secondary objective of the study was for evaluating the influence of fruits and vegetables wastes on plants when they were used as fertiliser (using digested and undigested sludge). However, all wastes were pre-treated using sieving machine for obtaining powdered samples which were then stored in a separate black polyethylene bags. The study concluded that, the difference in the production of biogas from each substrate, to a large extent depended on the nature of the substrate. It also concluded that, all five substrates used in this study were good substrates in producing biogas. Scano, et al. [280], cited numerous research conducted on biogas production from anaerobic digestion of fruit and vegetable wastes (FVWs), while very few obtained results from FVWs as a single substrate. The majority of those few studies were carried out using lab scale reactors. A study [32] was carried out on three

agricultural products and food waste (maize silage, fresh grass and potato waste) to produce and optimise biogas yield. Beating pre-treatment as a mechanical pre-treatment was applied on these three substrates. The study concluded that, beating pre-treatment of the three substrates is effective, therefore the application of a beating pre-treatment to other lignocellulosic materials was suggested.

2.8 Digestate

Digestate is the other main product of AD. It contains all materials that not digest during the digestion process. As aforementioned, digestate typically consists of valuable nutrients in varied amounts based on the feedstock. It can be either used as is or processed and used in multiple ways such as: a source of animal feeding, organic fertiliser, etc. [3, 34]. According to Weiland, [211], the digestate is considered as an improved fertiliser in term of its availability to crops and has the potential to substitute mineral fertilisers. It is usual that AD process results in a decreasing in the C/N ratio and in a mineralising of organically bounded nutrients such as nitrogen. Where, these two effects contribute in increasing the short-term N fertilisation effect. However, the typical food-based digestate contains the following three main nutrient elements: Nitrogen, Phosphate and Potash. In addition to that, digestate also contains of other small amounts of other nutrients and trace elements which can help in maintaining soil fertility. However, several farms which rely on traditional fertiliser have recently started using digestate as a bio-fertiliser. From an economic point of view, a farmer can achieve a significant saving in using digestate instead of traditional fertiliser. For instance, Gask farm is one of the farm-scale pioneers of the technology in Scotland. The farm used to spend £52,000 per annum on fertiliser. After they turned to use digestate, a huge saving has achieved and their spending reduced to approximately £10,000 per annum [56]. However, the uses of digestate in agricultural or in the other applications can contribute to the transmission of infection. Due to that and in order to save the environment, many countries are regulating the uses of digestate [198]. The composition of digestate depends mainly on the composition of feedstock. Off-farm materials like industrial organic leftover, biodegradable fractions of municipal solid waste, sewage sludge, etc. may contain various amounts of biological, chemical and physical hazardous materials. The existence of these materials in the digestate applied to a cropland or others can cause a harm for animal and human health and contribute in

environmental pollution. Moreover, prior applying the digestate in any application, must be carefully analysed. Storage and application of the digestate must comply with the national regulations and quality controls in each country [35]. Moreover, the Environmental Protection Agency (EPA) is the government agency responsible for protecting the environment in Ireland. This includes regulating and controlling the use of the digestate, sludge and others in agriculture to prevent harming animal, human health. The code of practice for the use of bio-solids in agriculture is the applied guideline for farmer and others in the Republic of Ireland for the uses of bio-solids in agriculture. The code is produced in response to the European Commission directive 86/278/EEC for protecting the environment and particularly soil when applying sewage sludge in agriculture [281].

2.9 An Overview Of The Integration Approach

There are a high diversity of biomass feedstock sources such as: sugar- or starch rich crops, lignocellulosic biomass, algae, etc. Investigating the potential of more biomass, offers the opportunity to explore and produce a wide range of new polymers and bio-based products. Starch rich crops, like maize, can be applied for producing several chemicals and bio-fuels [47]. Lignocellulosic biomass is a very abundant source of feedstock, which can be applied to produce various bio-based products/chemicals and bio-energy. It has a potential to meet the worlds demand of renewable energy and bio based products [77].

As is well known, the final major output of AD is a biogas. The production of biogas alone from such a process is not economical competitive due to the volatile of fossil fuel price indexes [3, 27]. Despite the significant effectiveness of AD, it would lead to major issues if it has been applied more widely, as the quantity of the digestates which would be generated are relatively high, with improper handling of the digestate causing serious environmental issues. In order to overcome these issues, plus maximising the profit of the process and minimising the waste streams production, attention has increased in the application of the bio-refinery concepts using AD process [25, 26, 244]. The bio-refinery concept is similar to the present petroleum refinery. It involves processing of biomass to produces multiple bio-products including chemicals, fuels, polymers etc. from organic feedstocks. In other words, it is a facility that integrates biomass conversion processes and equipment in

order to generate feed, food, fuels, value-added chemicals and energy from biomass [3]. Figure 17 below shows a simplified diagram of bio-refinery concept. This concept has received some research, but more study must be performed before an established system can prosper [43].

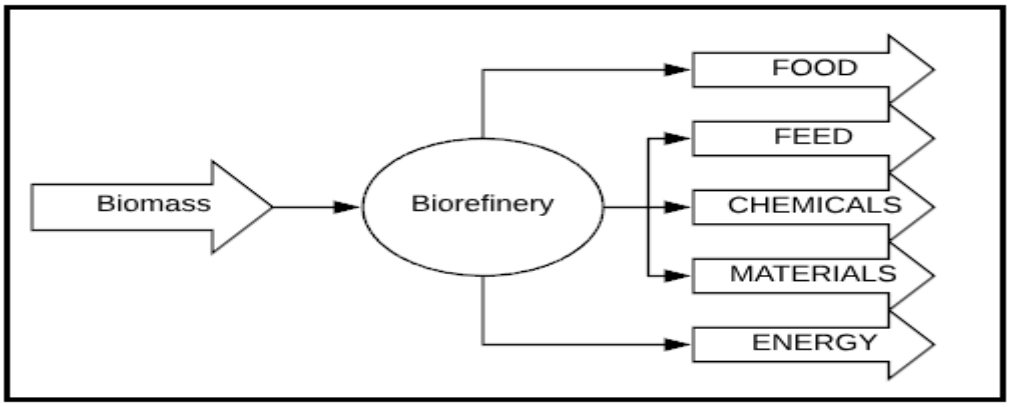


Figure 17: Simplified scheme of a bio-refinery concept [282].

An integrated approach aims to optimise its profit by producing low volume high-value products, meeting the energy needs through the production of low-value high volume fuels and minimising the waste streams generated. This approach corresponds to bio-refinery concepts and has recently gained much interests in many countries around the world [42]. Furthermore, there are three different types of bio-refinery discussed in literature; phase I, phase II and phase III bio-refinery.

- Phase I: Includes single feedstock, process and major product. There are many phase I bio-refineries in Europe for the production of biodiesel. Where, vegetable oils are used as a single feedstock in order to produce fixed quantity of bio-diesel and glycerine, as well through transesterification as single process.
- Phase II: Includes single feedstock and multiple processes and major products. This phase has the potential to produce major products such as: energy, chemical and material. The Nova-mont plant in Italy is one of the bio-refineries applying phase II. They are utilising corn starch as single feedstock to produce multiple chemical products such as: starch-based thermoplastic and biodegradable polyesters.
- Phase III: It is capable of using various types of feedstocks to produce multiple products (energy, chemicals and materials) or even various industrial products

through several processes [283-285]. Based on the feedstocks being used, phases III is further divided into four bio-refinery systems, such as:

A. Lignocellulose feedstock bio-refinery: it employs natural dry Lignocellulose materials (i.e. straw, corn, wood, stover, etc.).
B. Whole crop bio-refinery: it employs cereals (i.e. maize and wheat).
C. Green bio-refinery: it employs natural wet materials (i.e. green grass and algae).
D. Two-platform concept bio-refinery: the feedstock in this system can be separated into a sugar platform (biochemical, i.e. AD) and a syngas platform (thermo-chemical, i.e. gasification). The two platforms have the potential to generate energy, chemical and material, food and feed, therefore, exploiting full use of its feedstocks [42]. Figure 18 below shows a simple schematic of the two- platform concept bio-refinery system.

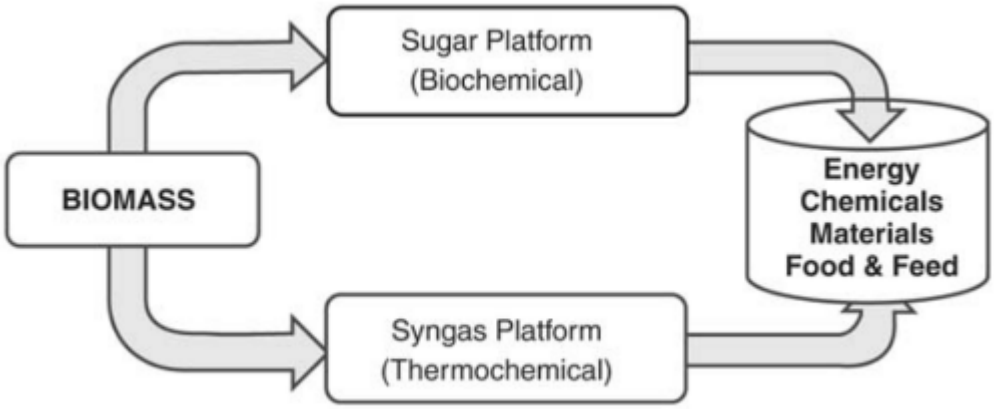


Figure 18: Schematic diagram of a two-platform bio-refinery concept [42].

In the design process of an integrated approach, three major principles must be adhered to: (a) all feedstocks will be applied are derived entirely from biomass, (b) all end products must be biodegradable, and (c) all processes must be carried out based on Green Chemistry and Clean Technologies [282]. In addition, the feedstock which will be utilised along with the end products must be carefully selected as well. A number of factors should be taken into accounts during selection, such as; the availability, feedstock's composition and its potential use in multiple production streams [286].

Basically, the final output of the AD process is biogas and digestate. The digestate or the effluent from the AD process generally consists of solid and liquid

streams. These two streams can be separated from each other and utilised in various applications. Ammonium and phosphates are the main nutrients that exist in the liquid digestate. Liquid digestate is widely used in agriculture as fertilisers. While, solid streams consist mainly of suspended solids and indigestible materials. Digestate can be also used as whole digestate (without separation). Actually, the composition of solid stream varies based on the substrate being used and the operating condition of the digester. These solid residues can be composted, used for dairy bedding or applied directly to cropland. It can be also used in making of a large variety of high-value bio-based products through bio-refinery concepts i.e. bio-plastic. [3, 43, 287]

The anaerobic bio-refinery is one of the most promising bio-refinery concepts. The majority of bio-refinery concepts studies describe and discuss bio-refinery concepts in general. Only a few studies have described the concept of anaerobic bio-refinery. In an anaerobic bio-refinery concept, bio-digester serves as a centrepiece for the bioconversion of feedstocks into several high-value, but low volume products (i.e., chemicals and drop-in bio-fuel) and high-volume with low value products (i.e., electricity, and conventional transportation bio-fuel). Promisingly attention towards anaerobic bio-refinery concept is now increasing and the use of AD as the final disposal step is desired [3]. Figure 19 provides an illustrative example of a large-scale anaerobic bio-refinery in order to produce bio-fuels and bio-based products.

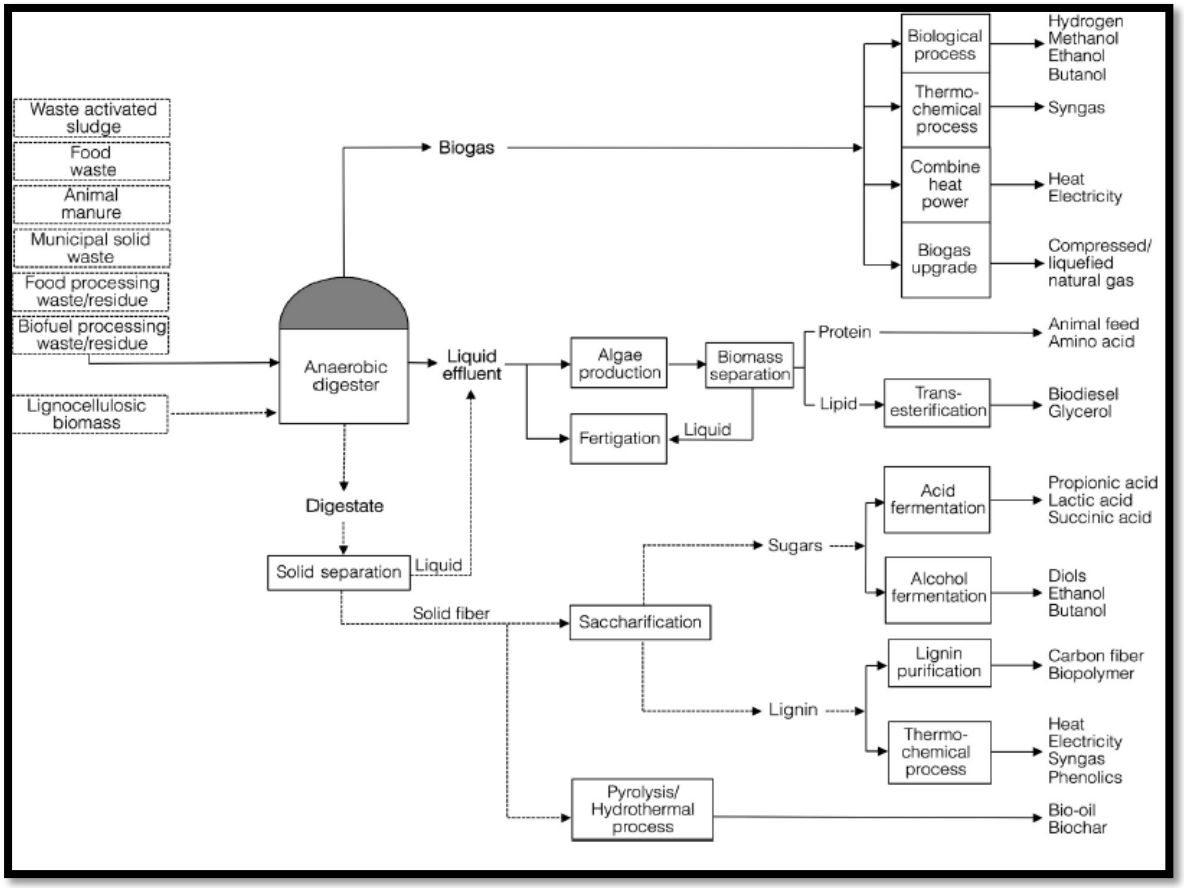


Figure 19: Schematic of a large-scale anaerobic bio-refinery [3].

2.10 Summary Of The Key Findings

The review of literature has been conducted extensively to cover all relevant research of the proposed research. From the literature review, it has been concluded that, the studies are still ongoing to discover more renewable and sustainable alternative energies for non-renewable energies due to its negative impacts on the environment, humans, animal, etc. AD is a bio-conversion process used to convert biomass as a bio-energy resource into biogas. Although this process has proved its effectiveness in converting many types of biomass, investment is still not economically attractive due to the operational, economical and environmental challenges associated with AD when it is applied at large scale. According to the literature, these operational issues can be avoided if they have been taken into account in advance. While, the economical and environmental issues still require radical solutions, investigating the major common issues and obstacles facing AD plants could significantly help emerging countries and the countries which intend to

invest in this field, solving or avoiding the issues in advance and making the investment in AD more desirable.

Beating pre-treatment as a mechanical pre-treatment method of biomass has demonstrated its potential in the pre-treatment of algae and other biomass. This method has not been widely utilised and requires more study to investigate its potential in the pre-treating other biomass such as: fruits and vegetables residue, etc.

Conventional plastic is one of the products derived from petroleum resources which is heavily used nowadays in all aspects of life. These petroleum products; (a) need thousands years until they are totally degraded, (b) have serious negative effects on human and all living organisms including marine animals and, (c) are considered as one of the main sources of environmental pollution. Therefore, there is a crucial need to find alternative bio-products that can be used in daily life. TPS has received more attention and it is anticipated to be a promising alternative for the conventional plastic. It is not yet completely favoured in industrial applications because of its poor properties. Thus, the studies are still continuing to improve its properties, quality and others through an addition of reinforcement materials.

Several types of digestate generated from different feedstocks have the potential to be used as a fertiliser, due to their contents of the main nutrient elements which traditional fertiliser contain. In order to prevent harming human, animal and the environment, the use of the digestate in agriculture are regulated by the government in many countries. In comparison to the current traditional fertiliser prices, a major saving can be obtained through the substitution of traditional fertiliser with digestate fertiliser. Due to this, its quality, etc., the substitution of the traditional fertiliser by digestate fertiliser is gaining a lot of attention nowadays and projected to increase even further.

Furthermore, an integration approach corresponds to the refinery concept. It is able to provide ideal and radical solutions for AD by incorporating the production processes and equipment to produce multiple bio-products from the whole biomass with very low or no wastes. Several studies have investigated the integration approach and more studies are still ongoing to exploit more biomass and therefore, discovering and producing more by-products and bio-products.

Additionally, there are many fruits and vegetables residues containing varying amounts of starch. Potato is a starchy vegetable, considered one of the major sources of starch just like maize, cassava, rice, etc., and its residues contain starch. Mango and avocado residues such as: peel, seed, seed coats constitute a large part of their fruits and are usually disposed of. The seeds of green mango and green avocado have high amounts of starch, before they are converted into sugar during ripening stage. The starch in the potato peel, green mango seed and green avocado seed can be separated and used as raw materials in several bio-plastic applications. In the proposed study, the residues of the potato, mango and avocado were selected for this study as they: a) contain starch in their residues, b) are highly consumed fruits and vegetables worldwide, c) provide more sources of starch and bio-fibre therefore exploit their applicability as raw materials in the production of bio plastic, and d) provide the possibility of combining them into one approach. In the Kingdom of Saudi Arabia, the residues of these three fruits and vegetables are not exploited and are usually disposed in very high quantity to landfill. Investigating the production of the AD biogas and bio-plastic from these three residues may attract investors to exploit these residues to produce bio-products. This would also encourage researchers in countries such as: the Kingdom of Saudi Arabia and others to study the potentials of other types of foods and attract more investment in its future.

Therefore, this study focuses on finding an optimal alternative to bio-energy and thermo-plastic starch composite for non-renewable energy and conventional plastic respectively from the residues of potato, mango and avocado. This is accomplished in the study through the incorporation of the AD process with the production process of TPS composite in an integrated approach. The approach aims essentially to solve the AD economical and environmental issues, through: (a) producing multiple bio-products to increase the economical feasibility of the AD process and therefore make its investment more attractive, (b) making full use of the three biomass in the study and the digestate generated after AD in order to eliminate the AD environmental issues which lie in the accumulation of the huge amounts of digestate. This mainly to economically investigate the influence of the incorporation of both processes on the AD. The next chapter describes the materials, equipment

and procedures that have been used and applied, to achieve the objectives of the study.

Chapter 3 : EXPERIMENTAL MATERIALS, EQUIPMENT AND PROCEDURES

3.1 Introduction

This chapter presents the aims of the laboratory experiments, materials and equipment used and the methodologies used during the experimental work of this research. They are described in the chapter within five main sections. Figure 20 illustrates the structure of the chapter.

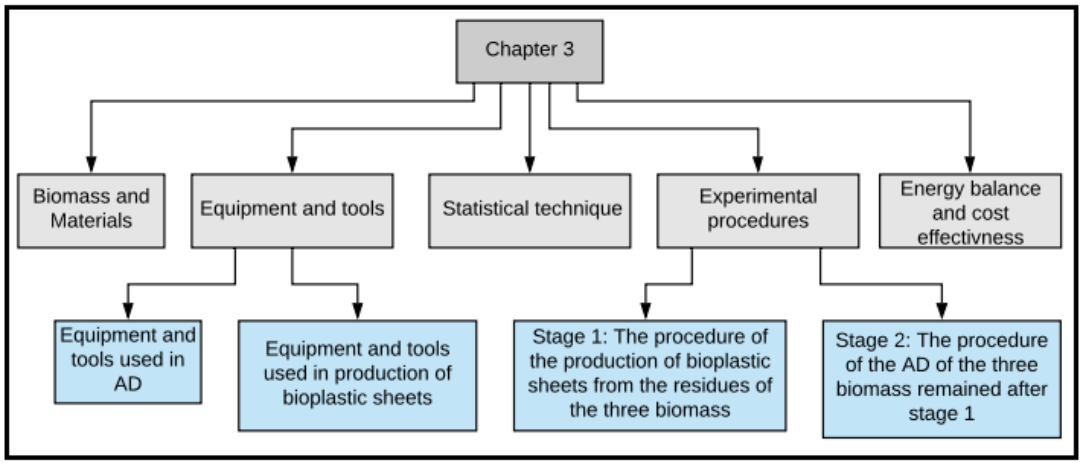


Figure 20: The schematic outline of this chapter.

The "Design of Experiments" (DOE) technique was selected to design the processes, statistically analyse the experimentations and optimise the results. This technique was applied successfully in a number of previous of studies on AD process to enhance the methane yields and others [32, 33, 185]. Furthermore, the primary objective of experimental works was to design and implement an integration approach that incorporate the AD process with the production process of the bio-plastic/mango fibre composite sheets based on the isolated starch, with the aid of the Hollander Beater. As already stated, this was accomplished in order to find a radical solution to the environmental and economical issues associated with AD and therefore proving that investment in AD is more desirable.

As it is described in Chapter 2, there are a number of factors that influence the AD process. In this study, the influences of the temperature, organic concentration and sludge concentration were evaluated and optimised. The influences of the temperature and sludge concentration were essentially considered because they represent expenses associated with the AD. On the other hand, the organic concentration was studied and optimised in order to balance the amounts of the substrate and sludge inside the digesters and to find the maximum amount of

organic that can be added into the digester and therefore to increase the contribution of AD in the waste management system. The research has also measured the (N, P and K) contents in the resulting digestate. This was achieved by confirming its bio-fertiliser potential and therefore, increasing the number of bio-products produced and limiting the accumulation of the digestate post the AD process. In addition, several properties of the bio-plastic sheets were examined to investigate their quality and price. The potential of the mango seed coat as reinforcement material (bio-fibre) in the production of the bio-plastic sheets were evaluated. The properties of the bio-plastic sheets produced were also optimised. The objective of the optimisation processes was to economically evaluate the incorporation of the TPS production process on the economic aspects of AD. This was accomplished by calculating the energy balance of the AD process and the cost effectiveness of the production process of the bio-plastic sheet based on optimum results at an optimum set of factors.

"Making full use of biomass" as a concept of bio-refinery was met by separating the starch from the three biomass and the mango seed coats and by using them as raw materials in production of other bio-products along with the biogas and bio-slurry.

Starch and mango seed coats might have positive influences on the economic aspects of AD. Therefore, the study compared between the quantity (biogas volume) and quality (the concentrations of the CH_4 and CO_2 in the biogas produced) of the biogas produced from each biomass before and after separation of the starch and mango seed coats. Whereas if they have a greater positive impact on the economic aspects of AD, then the incorporation will be not economically feasible.

3.2 Biomass And Materials

3.2.1 Biomass

1. Potato

Potato peel is the only residue of potato. Golden wonder potatoes which are also known as Russet potatoes is a common type of starchy potato. Among all types, it can be distinguished by its dark brown peel. Russet's flesh is white, dry, and mealy. However, the peel of the Russet potato was used in the study as one of the

raw materials for producing bio-plastic sheets, biogas and bio-slurry. A specified quantity (approximately 45 kg) of Russet potato was sourced from a fruit and vegetable wholesale market in Dublin city, Ireland. After washing and peeling them, the peels as the only residue of potato were pre-treated to isolate the starch. Appendix A shows the isolated peels. The pre-treated peels after isolating starch were further used as the substrate for AD process to produce biogas and bio-slurry.

2. Mango

Pre-determined quantity (approximately 25 kg) of a popular mango variety named Kent, were collected from a local shop in Dublin. Mango residues weight constitutes a large part (approximately 33%) of the total fruit weight. Peel, seed, seed coat are the main residues of the mango fruit. In order to make full use of all residues of mango; the starch was extracted from the mango seeds while, the fibres were separated from the mango seed coats (see Appendix A) and blended as reinforced material with the starch to produce bio-plastic composite sheets. The pre-treated peels and the leftover materials of the seeds were used as the substrates for the AD of the mango.

3. Avocado

A specified amount (approximately 20kg) of a common green cultivar of avocado named Fuerte was sourced from local supermarket in Dublin. Avocado peels and seeds which are normally disposed of were used as the residues of avocado. Appendix A shows the avocado used and its residues. In the study, the avocado seeds and peels were processed by the Hollander Beater. The starch separated was used to produce the TPS, while the remaining materials were used as the AD feedstock for the avocado residues.

3.2.2 Materials

1. Sludge

The sludge used in the AD process was dark brown tending towards black, heavy, with a high viscosity and unpleasant odour. It contained the micro-organism required for the fermentation process. The concentration of the sludge was investigated by studying its influence on the quantity and quality of the biogas produced and optimised in the study to obtain the optimal level of sludge. The

application of the sludge was mainly to provide the required "broad trophic" microbial composition in the digester.

A specified amount of digested sludge for each AD process in this study were sourced from the Green Generation limited plant in County Kildare, Ireland. The Green Generation plant is regulated by the Environmental Protection Agency in Ireland (EPA). All processes, inputs and outputs inside the plant are complied within the specifications and standards required by the EPA. The biogas produced from the plant is used as a fuel in CHP units, while, the processed digestate is used as a bio-fertiliser in farmland. Random samples of the sludge and digestate are periodically tested in an accredited Chemistry Lab. to ensure their quality. The composition of the sludge is crucial to be known in regard to the pathogenic organisms in the culture and the potential of the sludge for producing biogas. So that, the pH level of all sludge collected from the plant were measured and found to equal to a pH 7.9 ± 0.1. In addition, the results of the measurements of the dry matter and the compositions of the nutrients in the sludge and digestate were obtained from the plant. Appendix B illustrates the results of the tests the green generation plant measured in the Chemistry Lab. to measure the nutrient contents and dry matter that both the sludge and digestate contained as well as some certificates have been awarded to the Green Generation plant.

2. Plasticiser

Propan-1, 2, 3-triol or as it is well known 'Glycerol' with a purity ≥ 99.5% was applied to the starch in liquid form as a plasticiser. It was supplied from Sigma-Aldrich company, Co. Wicklow, Ireland. The amount of glycerol added to the starch is similar to the amount used in previous studies (30% of starch initial weight) [103, 104, 110]. Glycerol as a plasticiser converts the starch into thermoplastic and therefore facilitates extruding, injecting, moulding and pressing, in other words, it increases the flexibility and applicability of the plastic.

3. Distilled Water

Distilled water is pure and contains less or no impurities compared to water. In this study, it was used in the manufacturing processes of the bio-plastic sheets. In addition, the amount of distilled water added to each sheet was 12% of the initial starch weight [104].

4. Chemical Compounds

Sodium metabisulfite ($Na_2S_2O_5$) and sodium hydroxide (NaOH) were two chemical compounds used in varied concentrations. They were obtained from Sigma-Aldrich company, Ireland. $Na_2S_2O_5$ is a good anti-oxidant. 1% w/v of the Sodium metabisulfite was applied to the starch and fibres each to prevent them from oxidization. While, NaOH is a highly caustic base. It was applied to the mango seed coats in a solution of a 5% at 90 ^{0}C for 2 hrs to ease its mechanical pre-treatment process.

3.3 Equipment And Tools

3.3.1 Equipment And Tools Used In AD

Figure 21 below illustrates the apparatuses and equipment which were utilised in the preparation of the samples for the AD processes. In order to clearly describe the equipment used in the AD processes, this section is further divided into sub-sections.

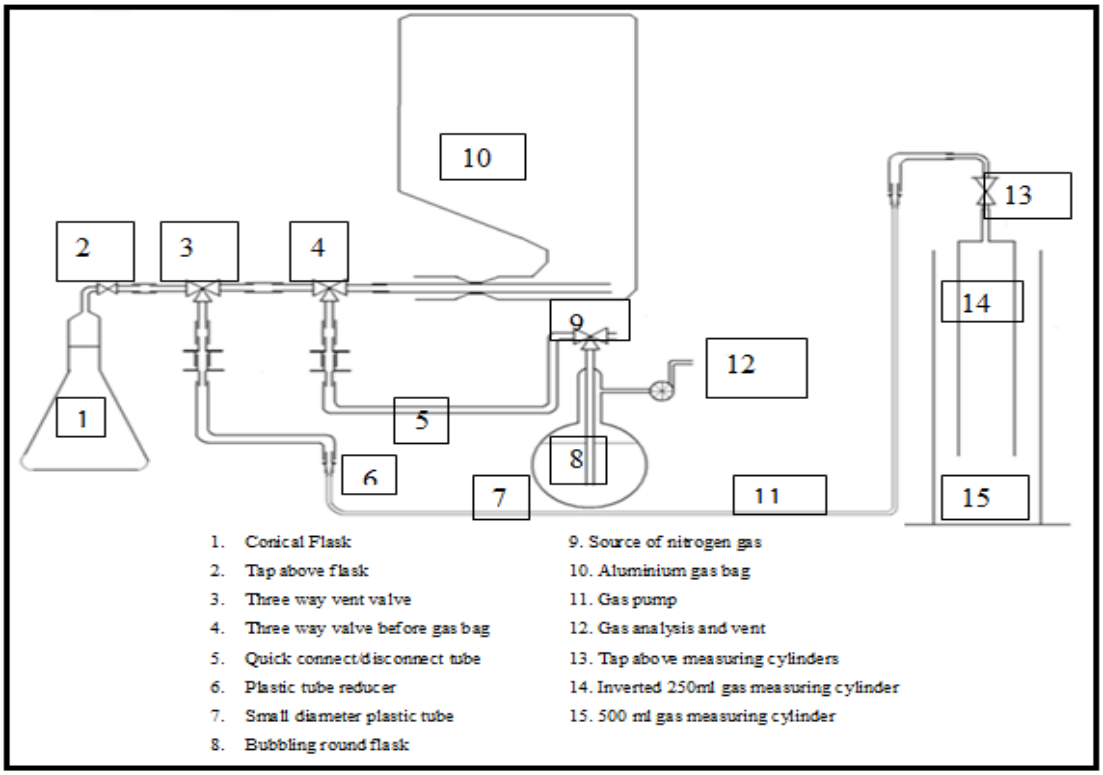


Figure 21: Apparatuses and equipment utilised in the preparation of the samples in AD.

1) AD Process

a. Mechanical Pre-Treatment (Hollander Beater)

The Hollander Beater is made up of mainly by a raceway (drum), a motor, and a V-belt. The raceway is filled with water and the biomass. It is equipped with a crank handle that allows the setting of the gap between the raceway's (drum's) blades and the bed-plate. The minimum gap can be obtained is 76 µm. It is corresponded to one single turn of the crank handle. It is provided with a small slot that can be opened/closed manually for drainage purposes and a scale to control the gap between the drum and the bottom surface of the device. This machine can perform two actions; (a) cutting action caused by the grooves located on the bed-plate, and (b) high pressure beating action of the feedstock against an inclined plate placed at the exit-outlet of the drum. Table 12 shows the technical specifications of Hollander Beater and Figure 22 illustrates Hollander beater and its main components.

The beater was mainly used to pre-treat the three biomass, isolate the starch from the potato peels and the seeds of the mango and avocado, and mechanically pre-treat mango seed coats to obtain the fibres required. This increases the accessible surface area of the biomass and reduces the degree of polymerisation of biomass used in AD process.

Table 12: The components of the Hollander beater device and its specifications [32].

Components	Specifications
Motor	• Wattage: 746 watts (1 hp) • Voltage: 220v • Ampere: 6.9 Amps • 1 Phase • Speed: 1450 rpm
V- Belt drive:	2.5: 1 Reduction
Tub	Volume: Max capacity =90L, and working capacity = 40L
Rum	• Speed: 580 rpm • Diameter: 200mm • Paddles: 24 paddles

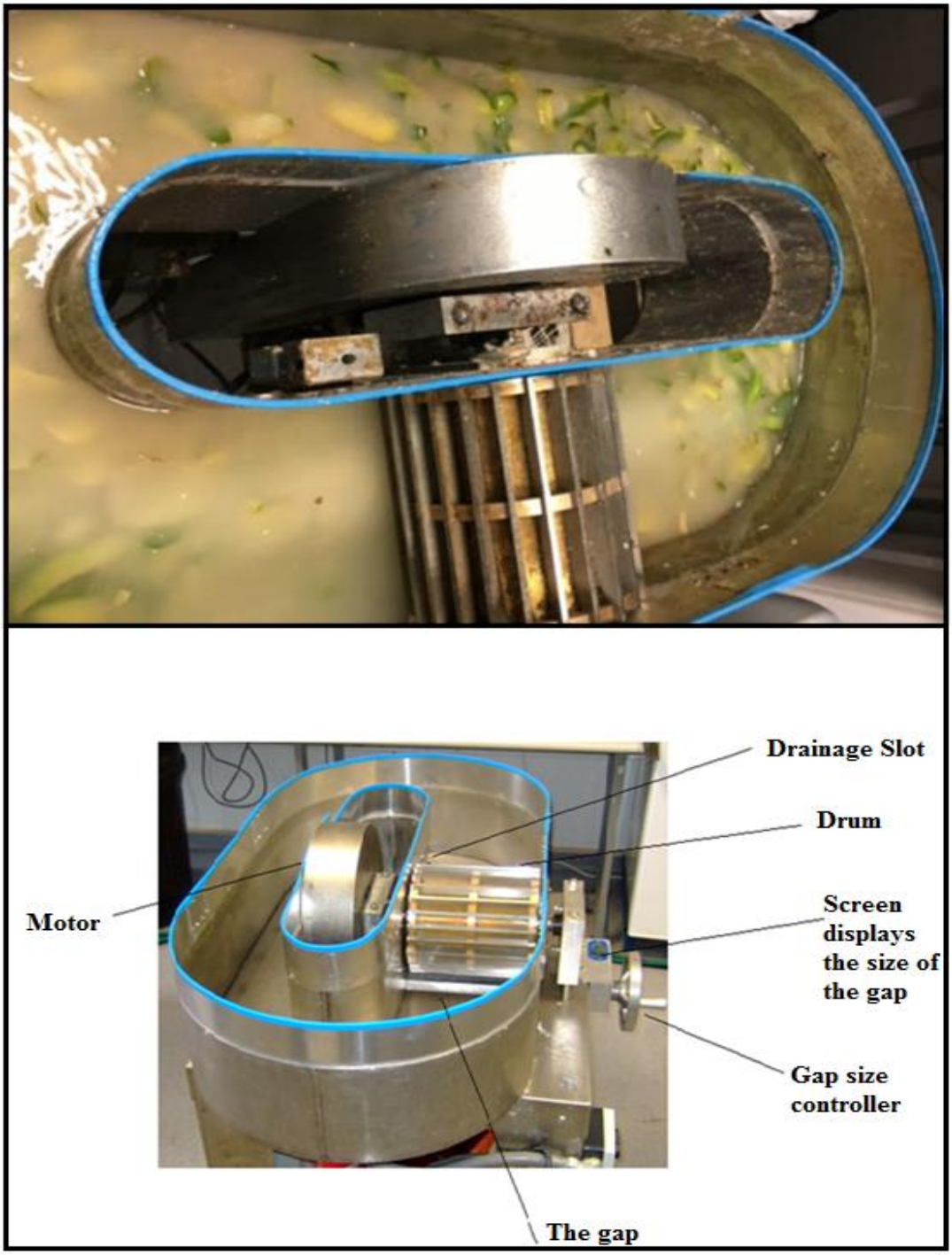


Figure 22: The Hollander beater.

b. Gas Collection Equipment

1. Water Baths

Five water baths were used in this study, and they were set at three different temperatures according to the design matrix. The water bath was equipped with a temperature sensor. It maintains the temperature of the samples at a constant level. All of the water baths were filled with water to their maximum level (Max. sign).

As the water in the bath heated up, it evaporated and the water level dropped. The evaporation of the water and therefore the drop of the level would result in inaccurate readings. For safety purposes, in order to avoid the dropping of the water level; to ensure the proper conduction of the experiment; and due to the possibilities of an irregular distribution of heat in the tank; the water baths were checked daily and filled up with water to the maximum level if required. Throughout the experiment, all reactors were checked daily and slowly mixed. Furthermore, the water baths were provided with plastic holders. The holders were tightened to two sides of each water bath to prevent movement of the reactor. Figure 23 shows the water bath.

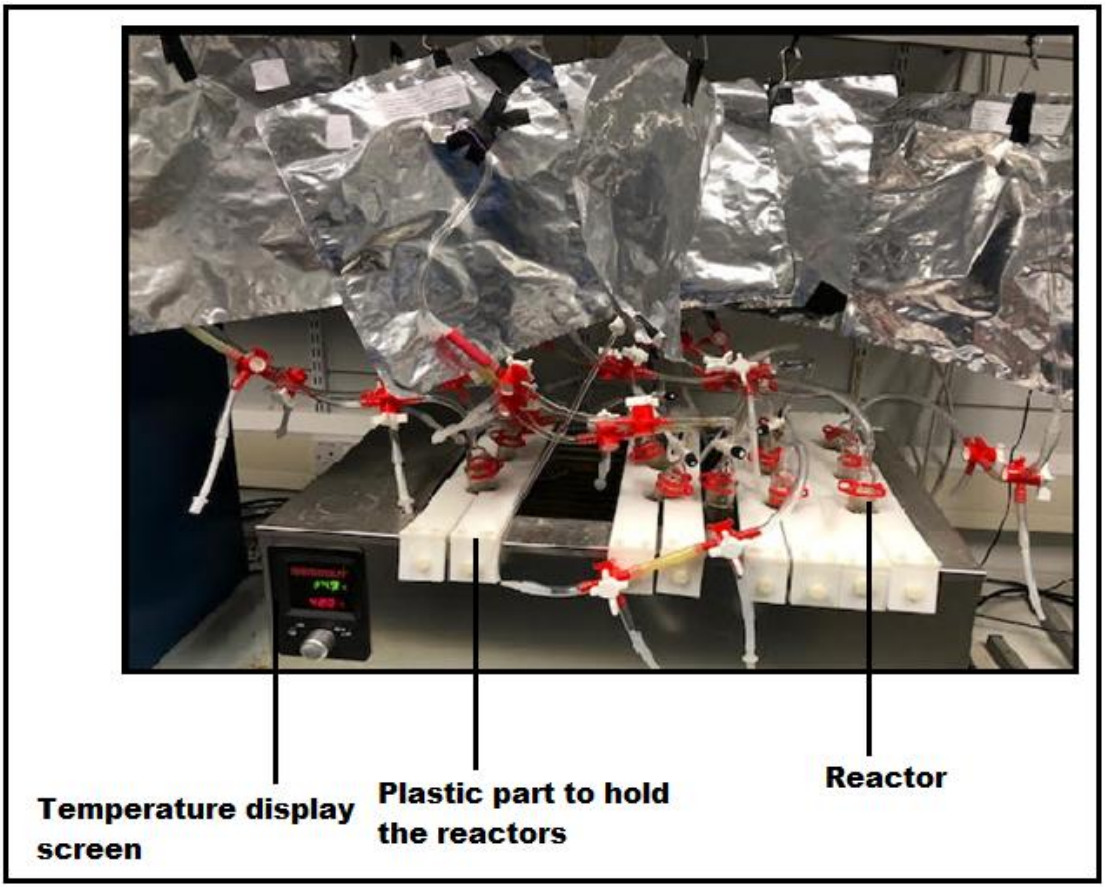


Figure 23: Water bath.

2. Electric Vacuum Pump

As the AD process must be carried out in an air or oxygen-free environment, an electric vacuum pump was used. It speeds up the process of extraction of the gas as well. In the preparation of AD reactors (equipment 11 in Figure 21), the gas goes

from the pump to a round bottom flask through a tube. Figure 24 shows the electric pump.

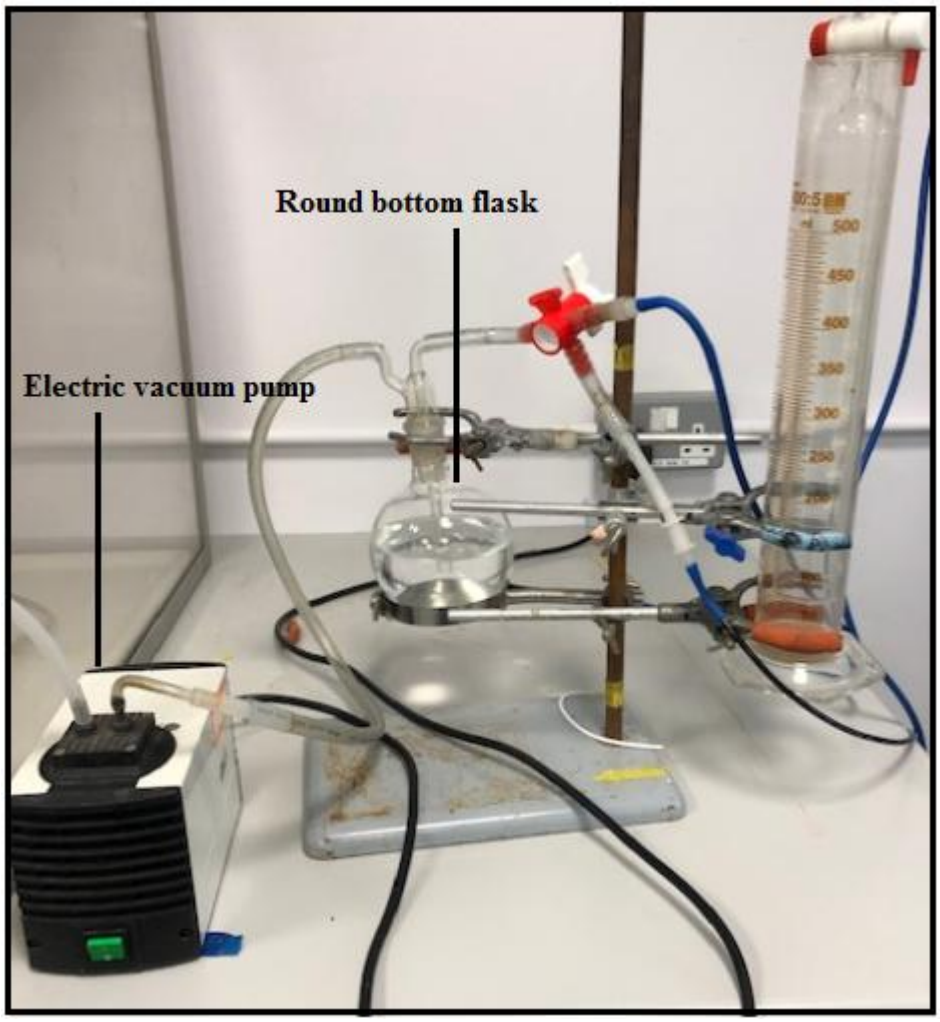


Figure 24: Electric pump used in the study.

3. Three Ways Valve

The three-ways valves shown in Figure 25 were used to control the movement of the biogas to and from the reactors and the aluminium bags. The three-way valve connects the reactors to the aluminium bag by connector tubes. Two three ways valves were attached to each gas collection system of a reactor. One of them for measurement and the other for purifying the systems from oxygen and other gases.

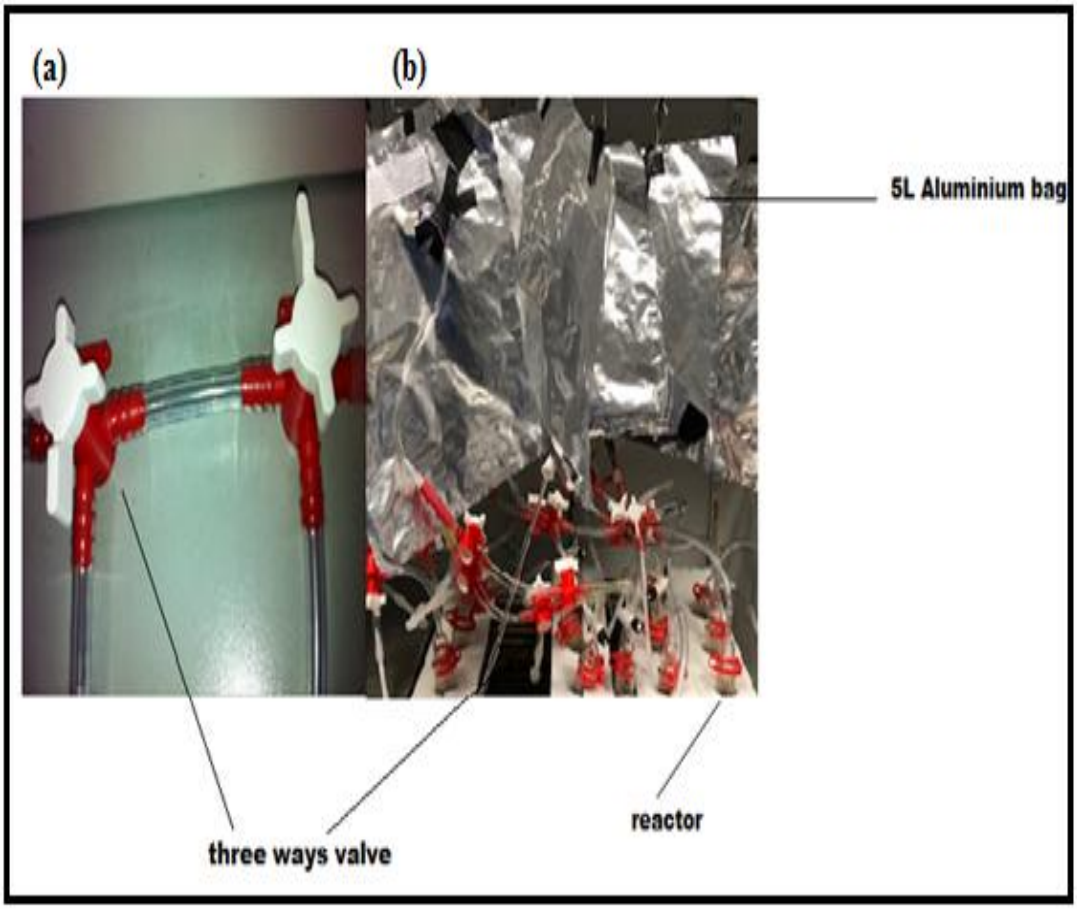


Figure 25: The three ways valve (a) and gas collection view (b).

4. Aluminium Bags

The aluminium bags are designed to store gas, made by Linde Company. In the experiment, each bag was connected to one reactor. The gas produced from the reactors were stored in these bags until they were fully collected. They were tightly sealed to prevent any leakage of gas, prevent entry of air into the bags and for safety purposes. In addition, the bags were labelled by codes to differentiate between them. Prior to the beginning of the process, each bag was ensured it is free of any gas traces (i.e. oxygen) by applying nitrogen (discussed later) to the bag. Figure 26 shows an image of the aluminium bags.

Figure 26: The Aluminium bags used in the study.

5. ***Nitrogen Gas Cylinder***

For anaerobic conditions, the fermentation process must occur in an air and oxygen free environment. Nitrogen gas was used to remove any gas present during the fermentation environment.

6. ***The Flasks Used***

- ***Round Bottom Flask***

Round bottom flask as shown in Figure 24 was used in the process of expelling oxygen from the system. The flask was connected to the electric pump by a tube. The electric pump extracted the gas and released it to the flask. The formation of bubbles in the flask indicated that, air was extracting out of the system. The water in the flask helped preventing air from flowing back into the system.

- ***Volumetric Cylindrical Flask***

A volumetric flask was used to measure the volume of the gas. To measure the volume of the biogas produced, the flask was filled with water to a certain level and the water level marked. An inverted 250 ml cylinder was placed inside the cylinder. The gas to be measured was thereafter pumped into the cylinder through a tube,

causing the water level to raise up. The volume of the gas was obtained by subtracting the initial water level from the water level after the gas was pumped into the flask. Figure 27 shows the volumetric flask and other auxiliary equipment used.

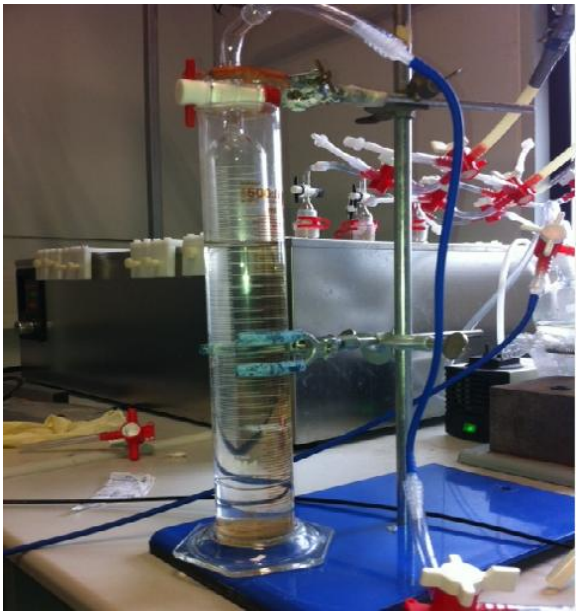


Figure 27: The volumetric flask used in the study.

- ***Cylindrical Flask***

The cylindrical flask shown in Figure 28 was used in AD to measure the TS%, VS% and the MS% of the biomass. It was filled with the biomass to a specific level and placed in the oven (discussed later) for specific time.

- ***Conical Flask***

In the AD process, conical flasks were used as the bioreactors or as is well known 'digesters'. All four decomposition stages in AD occurred (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) inside the flasks. Each flask was filled with a mixture of the pre-treated samples, water (if required) and sludge and placed in the water baths at certain temperatures. As can be seen in Figure 29, the flasks were attached to the gas collecting system by means of taps with glass bores.

Figure 28: The cylindrical flask.

- ***Other Glassware Used***

In addition to the glassware described in the AD, other glassware was used such as: beakers, pipettes, burettes, connecting tubes, and stopper caps. This glassware was used for the purposes of measuring, pouring the samples in the reactors, etc.

7. Red Clips

The red clips on the neck of digesters used are shown in Figure 29 and were attached to each conical flask (reactor) to clamp the flask with the glass bores and preventing leakage of gas.

Figure 29: Red clips, glass bores and conical flask.

2) Characterisation Equipment

1. Biogas Detector

The biogas detector shown in Figure 30 below was used in the experiment to measure the concentrations of the biogases produced: CH_4, CO_2, O_2, H_2S. Biogas 5000 detector is a digital biogas analyser manufactured by Geotech company. It is lightweight, sturdy, very sensitive with an accuracy of ±0.1 ppm and easy portable biogas analyser has been manufactured to provide accurate measurements for gas levels. The high-quality design of the analyser has made it applicable to be used in many hazardous AD sites. The analyser calibrates itself after each turning OFF/ON of it. During operation, it gives a warning message if there was an issue in the calibration. Additionally, after each run, the analyser is reset to expel any remaining traces of gases or air using the fan button in the analyser (see Figure 30). The calibration of the analyser was also checked periodically by the manufacturing company or when required.

Figure 30: Biogas detector.

2. Laboratory Oven and Furnace

The muffle furnace and oven were used in the study to measure the moisture content, total solid and volatile solids of each AD of the three biomass. The samples were taken from a container and heated in the oven at 105 °C until a constant weight (The constant weights were confirmed by obtaining the same weight of three measurements of the sample in one hour interval between each of them) was achieved (approximately 24 hrs). Thereafter, they were burned in the furnace for 4 hours at 575 ± 25 °C [288]. In the first 20 minutes of burning a sample, smoke would generate inside the furnace. After that, the amount of the smoke would gradually decrease until it became very low. The furnace is designed with a vent hole at the top to allow the smoke to escape. Thus, the furnace was placed in a fume hood to expel the smoke.

3. Electronic Weighing Scale

An electronic weighing scale was utilised to measure the weight of products. In the AD, it was applied to measure the weight of the biomass before they were placed in the beater and for working out the VS weight. In the bio-plastic sheet production process, it was used to measure the weights of the components of the composite (i.e. starch, fibres, and so on). After producing the bio-plastic sheet, it was used for measuring the weight of the specimen before measuring its density. Prior to measuring the weight of sample, the weights of the beaker or container (where the sample was placed) were measured by the electronic scale in order to obtain the exact product weight. The electronic weighting scale used has a range of from 0 to 11 kg and an accuracy of ±0.1g.

4. pH Level Meter

The pH level is an important factor during the AD process. It determines the equilibrium of the system and the stability of the digester, as the pH level can vary in the concentration of the gases produced therefore, the pH levels of all samples were measured before and after the process. Hanna Instruments pH 211 Microprocessor pH Meter (shown in Figure 31) was used to measure the pH levels (accuracy ±0.01 pH). Prior to using it in each sample, the calibration of the meter was checked and calibrated if required. The meter was calibrated and adjusted by measuring the pH of a solution has a known pH. The meter was equipped with a probe. When measuring the pH, the probe is inserted in the reactors, ensuring the

probe end is fully immersed in the sample. The probe was left in the sample until the pH level reached constant. Thereafter, the probe was wiped, cleaned and placed in a tube filled with water.

Figure 31: Hanna microprocessor pH meter.

3.3.2 Equipment And Tools Used In Bio-Plastic Production

1. ***Electric Heater***

An electric heater is a common device used in laboratories. It is powered by electricity and generates heat. The heater has a control switch to stir the solution by a magnetic stirrer placed in the beaker and another switch to set the temperature. It is used in the experiments to heat up the solution of NaOH and mango seed coat at 90 ^{0}C for 2 hrs. The heater has a capacity of 600 Watt, maximum temperature 300 °C and 2800 rpm. Figure 32 shows the heater used in the present study.

The Laboratory magnetic stirrer was used for stirring the solutions. It is commercially available in varied lengths. The magnetic stirrer was placed in a beaker containing NaOH solution and mango coats to stir the solution (see Figure 32 (c)).

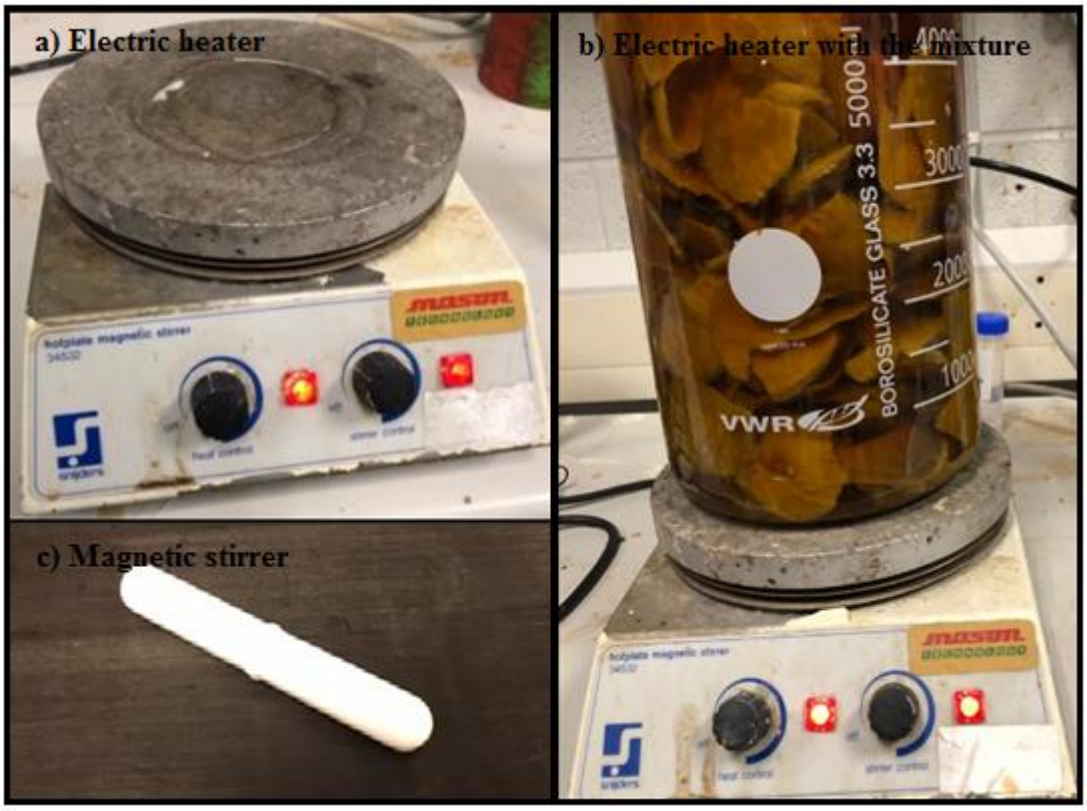


Figure 32: Electric heater stirrer (a), with mixture (b), and stirrer (c).

2. ***Energy Consumption Meter***

An energy consumption meter (Figure 33) was used to measure the electric energy consumed during the heating up process of the mixture of the bio-plastic sheets, beating pre-treatment and digestion process. As the water baths were adjusted to three different temperatures, three meters were used and each meter was connected to a water bath. The meter displays the energy consumed in kWh on an accuracy of ± 0.01 kWh.

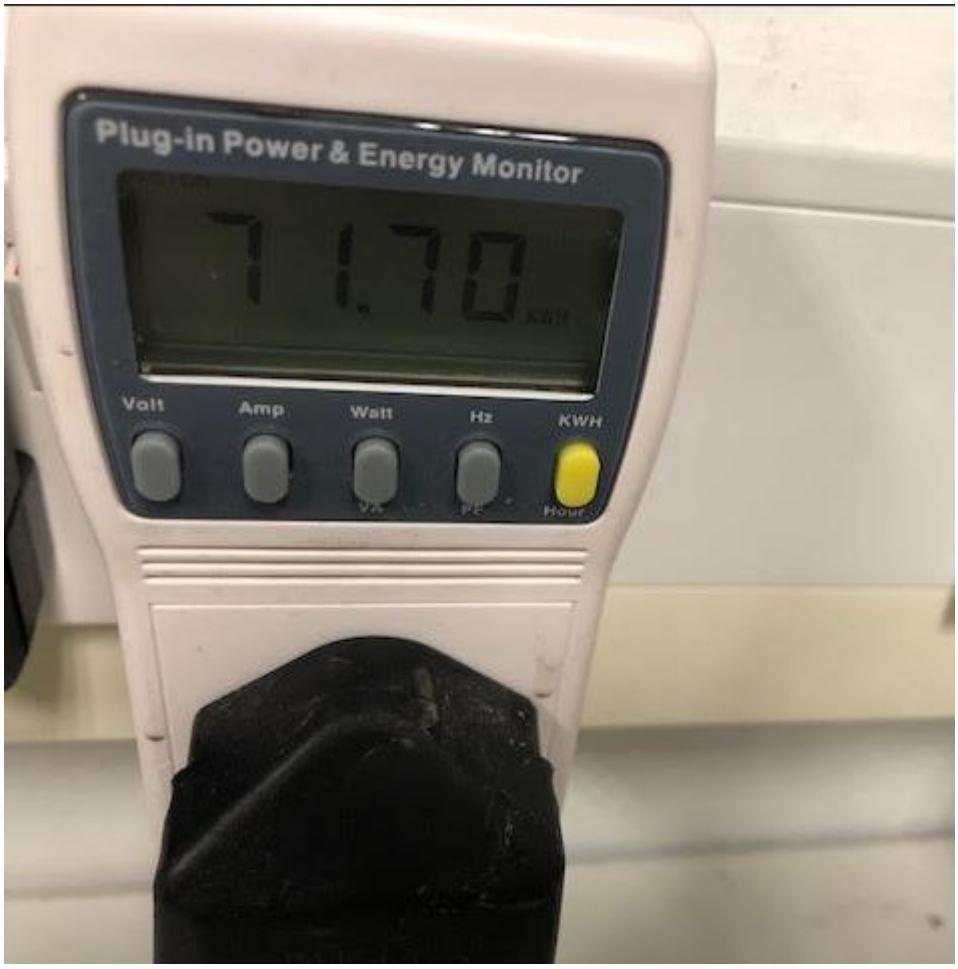


Figure 33: Energy meter used to measure electric energy.

3. ***Kitchenware***

A variety of kitchenware were used during preparation and testing. They were used for: peeling the fruits and vegetables, storing starch, storing the pre-treated residues until the digesters were fed with them, opening the seed coats, cutting the seeds into quarters, filtering the pre-treated residues to isolate starch, and purifying the fibres and starch.

4. ***Bio-Plastic Sheet Mould***

After preparing the starch/fibre blend, it was placed in a mould and processed. The mould was designed using of Solidworks software. Figure 34 shows the mould and the upper and bottom parts. Appendix C illustrates the design of each plate separately. The mould contains of two parts act like a male and female. The bottom part consist of three plates. The bottom one designed with a cavity for placing the heating elements. The middle one is a support plate for avoiding bending or breaking of the mould due to high compression, while the upper plate contained the

starch mixture. The upper plate of the lower part was drilled with a non penetrating hole from one side to facilitate measuring and adjusting the temperature of the mixture during compression. The diameter of the hole was determined according to the diameter of the probe (Ø= 3mm) of the thermo-couple. On the other hand, the upper part consist of two plates. The upper plate designed with a cavity for placing the heating elements into it, the bottom one acted as a male.

A parchment and greaseproof paper was used to simplify the de-moulding process of the bio-plastic sheets and avoid the sticking of the mixture to the plates. Prior to placing the mixture in the mould, two paper sheets were cut according to the dimensions of the plates and placed above and below the starch mixture. After producing the bio-plastic sheet, it was cut out according to the sample dimensions quoted from the International Standard methods of the proposed tests in order to test the properties of the material produced.

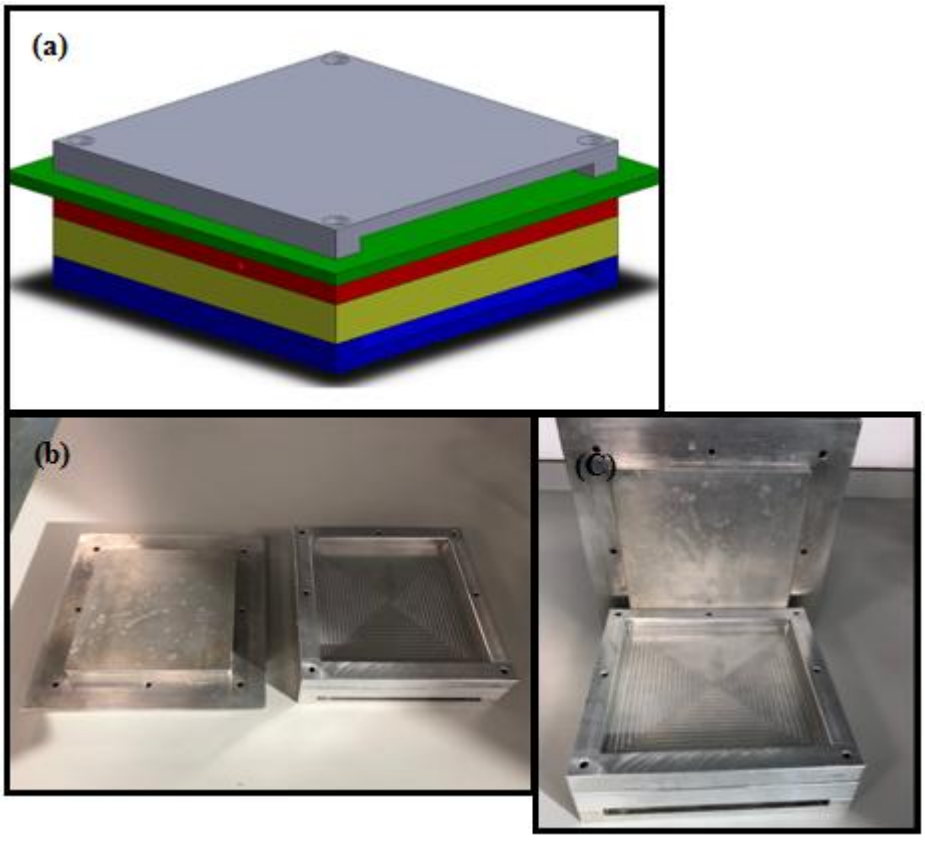


Figure 34: The Solidworks mould (a), and two view of final mould (b) and (c).

5. Temperature Controllers

Two PID digital temperature controllers were used to measure and adjust the temperature applied to the starch mixture. One of the controllers was connected to

four strip heater and the other one to a K-type thermocouple. Throughout the process of manufacturing the bio-plastic sheet, the thermocouple probe was inserted and left in the predrilled hole as previously stated, located in one side of the upper plate of the lower part (the Red plate in Figure 34 (a)) to monitor the temperature applied on the mixture. For safety purposes, the controllers (Figure 35) were placed in two separate Aluminium boxes.

Figure 35: Temperature controllers used to measure and adjust temperature during moulding.

6. Strip Heater (Mica Strip)

Four flat Mica strip heaters (Figure 36) with a dimension of 152.4 mm * 76.2 mm each were used, in order to distribute the temperature (140 °C ± 2 °C) evenly across the mixture, two heaters were placed in the upper part of the mould (the Gray plate in Figure 34 (a)) and the other two heaters in the lower parts (the blue plate in Figure 34(a)). As previously stated, the four heaters were connected to a temperature controller for controlling the temperature. Moreover, the heaters were connected to the electricity through an energy consumption meter to measure the energy consumed in the manufacturing process of the bio-plastic sheets.

Figure 36: Strip heater (Mica strip).

7. Electrical Blender and Coffee Mill

An electrical blender and coffee mill were used in the manufacturing process of the bio-plastic sheet. They were used with the pre-treated mango coats for a specific time to ensure the large agglomerations were separated from fibres into a small agglomerate and cut the fibres into shorter length.

8. Hydraulic Compression Machine

There are numbers of conventional processing technologies used in the manufacture of plastic. Compression moulding technology is one of those technologies. Unlike other technologies, compression moulding does not require the materials to pass through gates, sprues, etc. to fill in the mould cavities, as the, passing of raw materials through gates, etc. may require more consumption of the raw materials. However, compression moulding technology was used to manufacture the bio-plastic sheet by employing the hydraulic press (shown inFigure 37). The mould was placed on a base, and then the blend was compressed at a pressure of 34 MPa ±3 MPa, temperature of 140 °C ± 2 °C and load 75 kN ± 2 kN

for one hour. The press was equipped with an arm for pressing and de-pressing hydraulically. It displays the pressure applied in a pressure gauge. The blend was heated up by the flat strip heaters placed along the upper and lower parts of the mould.

Figure 37: Hydraulic press used to compress the polymer mixture.

A load cell device (accuracy ± 0.01 kN) was used with the Hydraulic press (Figure 38) to determine the average force applied on the mixture during the compression process. The compression of the mixture stopped when the lower plate of the upper part fully entered into the upper plate of the bottom part, i.e. when the mould was totally closed.

Figure 38: Load cell device.

9. ***TPS Composite Materials Cutting Machine***

Figure 39 shows the CO_2 Laser cutter engraving machine used to cut off the bio-plastic sheets produced. The machine was connected to an air extractor machine for expelling the cutting fume out. The bio-plastic sheets produced were cut based on the ASTMs for each of the tests, drawn using Solidworks software and defined on the machine through an USB. Three specimens were cut for each test for each composite sheet produced.

Figure 39: CO_2 Laser cutting machine used for each TPS composite material.

3.4 Statistical Techniques

3.4.1 Design Of Experiment (DOE)

The design of experiments (DOE) is a statistical technique provided by the Design Expert software. It helped in identifying the influences of factors and their interactions on the responses. DOE provides results both numerically and graphically. It helped also in finding out the optimal results, the optimal conditions and statistically analysed the data. The latest release of the software was applied in the study, with new and useful feature for ease-of-use, functionality, power and appeal. The application of DOE contributes in achieving major improvements to the process and identifying the optimal settings for the process in order to attain peak AD, etc. performance [32, 33, 289, 290].

3.4.2 Response Surface Methodology (RSM)

Engineers and scientists always wish to optimise the process they are studying. This involves determining the values of the input parameters at which the responses reach their optimum. RSM is one of the Design Expert technique available within its software. It is used in describing and assessing the performance of a process and obtaining optimum results.

RSM is a set of mathematical and statistical techniques that can model and estimate the responses of interest in which affected by various input variables, with a goal to optimising the process [103], it can be used to identify the relationships between the resultant responses and the input variables [104]. The Central Composite Design (CCD) and Box-Behnken Design (BBD) are the most popular approaches used in RSM. They are used in experimental designs to improve and develop second-order models. BBD uses 3 levels, coded as -1, 0, and +1. It has the potential to use any number from 3 to 21 parameters. They are generated by combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial analysis. As there are only three levels, a quadratic model is appropriate. On the other hand, CCD is the most popular design for a second-order modelling. CCD consists of three groups: two-level factorial or fractional factorial design points, axial points and centre points. CCD's predicts the coefficients of a quadratic model. All data are

in terms of coded values of the factors. In this study, BBD was applied for designing, analysing and optimising the AD process of each of the three biomass. On the other hand, mixture design as another approach was applied for the process of manufacturing bio-plastic sheets. This approach generates varied values to each component at each run of the process while maintaining the original total of the components. For instance, if the total weight of the mixture is 50 g, the design will generate specific number of runs (mixture of components) with a total of concentrations of each component at each run equal to 50 g. In this study, RSM and mixture design were used:

1. In the AD process, to determine the parameter and the interactions that have significant influences on the responses as well as the optimal settings of the parameters. While in the production of bio-plastic sheet, to determine the optimal bio-plastic mixture in terms of quality and cost.
2. Illustrating the developed models numerically and graphically.
3. Identifying the optimal set of the process input parameters, using both numerical and graphical optimisation, to obtain a specific target criterion.

RSM and mixture design are carried out in sequential steps. In this study, the following sequential steps were carried out to develop a model in the cases of the AD and the production of the bio-plastic sheets:

1. Finding the main factors of the process: The main factors can be identified by conducting a preliminary study (i.e. screening study) based on factorial design or partial factorial design. In the present study, temperature, organic concentration and sludge concentration were the influencing factors investigated. The main factors of bio-plastic sheet production process were 4 components. They were the weight of each type of starch and the fibre amount.
2. Finding the limits of each factor: The limit of each factor influencing the AD process was identified to be 3 levels and the levels of the bio-plastic sheets production process were as described in b) below.

a) AD: The levels of the temperature were set to be 32, 35, 38 ^{0}C, the organic concentration 1.6, 4.05 and 6.5 g-VS and the sludge concentration 20, 35 and 50 %.

b) Bio-plastic sheet production process: The total weight of starch and fibre in which applied in each run was 100g and the level ranges of potato starch was from 4 to 94g. The range of the quantities of the mango and avocado starch were set to be from 0 to 45 g each. Multiple preliminary trials implemented to adjust the range of the mango fibre amount. The trials revealed that, lower than 4 g results in a poor mechanical properties and appearance of cracks. While, higher than 10 g was seem to be too many as the mould did not fully close and applying of higher force could bend the mould. Therefore, the range of the fibre quantity was set from 4 to 10g.

3. Design matrix development: In the BBD design, the design matrixes are shown in coded and actual values (0, 1, and -1). While, it is shown in numeric values in the mixture design.

4. Performing the experiment: The AD processes and bio-plastic sheet production process were carried out according to the design matrixes. To avoid systematic error, they were arranged and conducted in a random order.

5. Recording the response

6. Development of mathematical model: The functional relationship represents any response of interest.

7. Estimation of the coefficients in the model: Regression analysis was used to calculate the values of coefficients. The software was used to evaluate the coefficients for the response of each experiment.

8. Testing the adequacy of the models developed: ANOVA helped in testing the adequacy of the models developed. The statistical significance of these models and each term in the regression equation were also tested using the sequential F-test, lack-of-fit test, and other adequacy measures (such as: R^2, Adj- R^2, Pred. R^2 and Adeq). In addition, the prob. > F (sometimes it is called the *p*-value) of the model and each of the term in the model can be computed by means of ANOVA. In terms of the R-squared, the value close to 1 means the reqression of the model is good. The lack of fit higher than the level of significance means, it is insignificant and the model adequately fit the data. The adeq precision is another

adequacy measure tool, it measure the noise ratio in data. Its value more than 4 means the model was able to navigate the design space.

9. Model reduction: If the whole model contains insignificant model of terms (i.e.: with a p-value greater than the level of significance α), it should be eliminated. This elimination can be done manually or automatically.

10. Development of the final reduced model: The final reduced model was developed at this stage. This final reduced model was only made up of significant terms, that make up the hierarchically structure of the model. A reduced quadratic ANOVA table can also be produced at this stage.

11. Post analysis: At this stage, it is possible to predict the response at the midpoints by using this adequate model. Creating plots, contours, and perturbation plots can demonstrate the effect of the factors on the responses. [291-293].

3.5 Experimental Procedure

The experimental procedures of the integrated approach was divided into two main stages (according to how the results are presented in Chapter 4):

A. First stage: the AD of the three materials remaining after separation of the starch and fibres.
B. Second stage: the production of bio-plastic sheets from the residues of the three materials. This stage involves properties tests of the sheets produced.

These stages are interconnected to each other. After collection of a specified quantities of each biomass, they were peeled, and the residues were separately washed with clean water, sliced into small pieces and prepared for the beating pre-treatment except the mango seed coats. The coats were further chemically and mechanically pre-treated. Figure 40 describe the flow chart of the integrated approach. The experimental procedures of each stage are described in the following sub-sections.

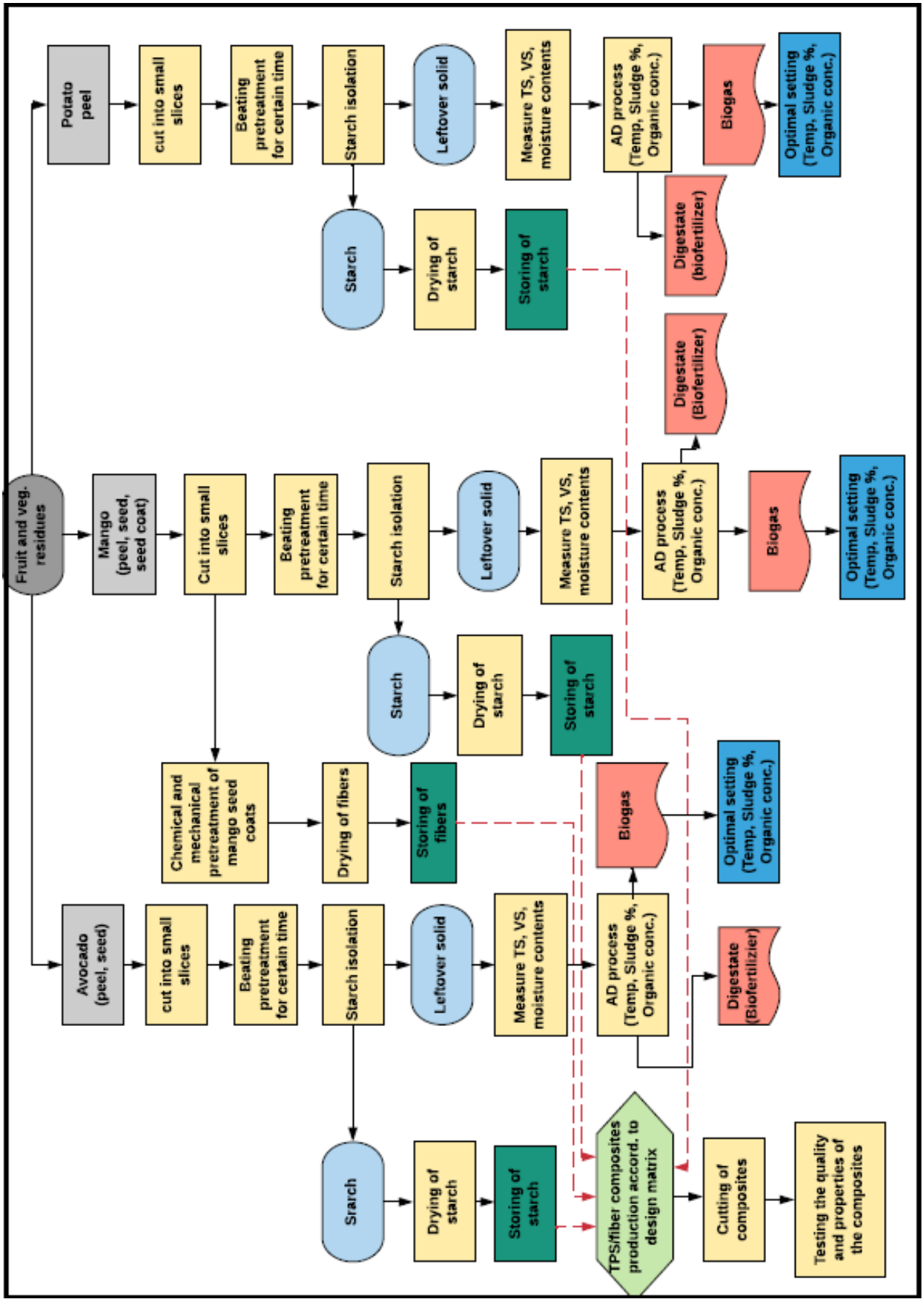


Figure 40: The flowchart of the integrated approach.

3.5.1 The Experimental Procedure Of The First Stage

The first stage was divided further into four steps. These steps are outlined in the next sub-headings and the procedures followed to conduct each of the four steps are described in detail in Appendix D. The raw materials of this stage were the three residues that remained after the extraction of starch and mango coats. Table 13 describes the AD design matrix. It was designed by RSM technique and applied to each biomass separately. As shown in Table 13, the temperature, organic concentration and sludge concentration were the factors investigated. The factors were optimised in order to obtain the optimal setting in terms of biogas yield and quality. Each factor had three levels represented as actual values in Table 13.

Preliminary trials were conducted before the AD to obtain the range of each of the three influencing factors. As aforementioned in Chapter 2, the AD process is more stable and require lower energy expenses at the mesophilic range temperature. Both psychrophilic and thermophilic temperature ranges could negatively influence the methanogenic activities. Due to that, the three levels of temperature were selected to be within the mesophilic range. Mesophilic is moderate temperature ranging from 25-45 ^{0}C. 35 ^{0}C was selected to be the central level of the range [216], while, 32, and 38 ^{0}C were assigned to be the border lines temperatures.

Organic concentration or as is commonly known VS concentration range were determined in reference to the total weight of all components in the digester (400 ml). The preliminary trials revealed that, the excessive feeding of the digester causes a failure to the system, while, insufficient feeding leads to a biogas low in quality and volume [227, 229]. Due to that and for facilitating the comparison of the results of the three biomass, the range of the organic concentration of all biomass were set to be from 1.6 to 6.5 g-VS.

The sludge concentration at its highest level, indicates that the sludge will be fed into the digester as it is (without dilution). While, when it is at its lowest level (20%), water dilution is used to adjust its concentration (100 ml sludge + 100 ml water). The attempts to specify the range of the sludge concentration revealed that, the concentration of the digested sludge lower than 20% of the total amount of all components inside the digester (400 ml) results in low CH_4% and high CO_2%, while the concentration above 50% lead to digester failure [227, 229]. Based on that and

according to the recent studies, the 20% and 50% were set as the border of the sludge concentration range and therefore, the three levels were set to: 20, 35 and 50%.

Table 13: The design matrix applied to each single biomass.

Std	Factor 1	Factor 2	Factor 3
	A: Temperature, °C	B: Organic Conc., g	C: Sludge Conc., %
	°C	g-VS	%
1	32	1.6	35
2	38	1.6	35
3	32	6.5	35
4	38	6.5	35
5	32	4.05	20
6	38	4.05	20
7	32	4.05	50
8	38	4.05	50
9	35	1.6	20
10	35	6.5	20
11	35	1.6	50
12	35	6.5	50
13	35	4.05	35
14	35	4.05	35
15	35	4.05	35
16	35	4.05	35
17	35	4.05	35

Step1: Starch and Fibres Separation

This step was carried out mainly for isolating starch and fibres from the biomass and make them ready for use, Appendix D shows in detail the experimental procedures of this step and the following three steps.

Step2: Measurement Of The MS, TS and VS Of Each Biomass

Figure 41 shows the pre-treated potato peels after they were dried for 24hrs and after they were combusted for 4 hrs.

Figure 41: The pretreated potato peels as obtained after dried (a) and combusted (b).

Step3: Anaerobic Digestion Process

In the AD process, all reactors were filled in with 400 ml of the pre-treated biomass, sludge, and tap water (if needed) in varied amounts according to the design matrix in Table 13. The organic concentration was adjusted by water dilution. All reactors were then placed in the water baths according to the design matrix. The retention time for all biomass was 21 days.

Step4: Measurement Of The Biogas Produced

Throughout the period of the process, the biogas was collected on days 7 and 21 to avoid leakage or burst of the aluminium bags. At each collection, the volume of biogas produced by each reactor was measured and recorded. The volumetric flask and the biogas detector were used to measure the volume of the biogas and the gases concentrations respectively. In order to evaluate the digestion of the organic matter and certify that the digestate contains of the basic nutrient elements of fertiliser, a selected digestate was sent to a chemistry laboratory to analyse its contents from the basic nutrients of fertiliser (N, P and K) and measure the remaining dry matter.

3.5.2 The Experimental Procedure Of The Second Stage

The procedure for the production of the bio-plastic sheets, was adapted and modified from several recent scientific literatures [16, 85, 103, 104, 110, 294]. This stage was designed by Mixture design. Table 14 shows the design matrix of this stage. The total weight of the starch and fibres in each run was 100g. The starch content in each sheet was either from potato starch as it is one of the popular and conventional starch types in industries or a mixture of starch derived from the three biomass at varied concentrations. Mango fibres were derived from the pre-treated mango seed coats. They were blended with each composite material according to the design matrix in Table 14 to improve the properties and the quality of the bio-plastic sheet. Potato peel was the single residue of potato which was considered the only vegetable biomass in the study. The two fruits biomass used were the mango and avocado residues. Mango residues included; the peel, seed, seed skin and seed coat while, avocado residues included only the peel and seed. The responses in this stage were the density and three mechanical properties: tensile strength, flexural stiffness, and impact strength. However, the process of producing bio-plastic sheet was implemented via two major steps: (a) manufacturing of the bio-plastic sheet, and (b) testing the bio-plastic sheet produced.

a) Manufacturing Of Bio-Plastic Sheet

According to Table 14, 20 bio-plastic sheets were produced and tested. Figure 42 shows the thermo-compression step of the production process. The same production procedure was followed for producing each sheet. The type and amount of starch in each sheet as well as the quantity of fibre are specified in Table 14. Three bio-plastic sheet were manufactured without fibres from the starch of each single biomass to evaluate the influence of the fibres on the quality and properties of the TPS. Two specimens were taken from two different area of two bio-plastic sheet to check the homogeneity of the starch and fibre using optical microscope. Figure 43 shows the three starch types were obtained from the previous major step (starch and fibres separation step) in the first stage. Appendix E illustrates the procedure was followed to carry out this step.

Table 14: The design matrix of the second stage.

Run	Component 1	Component 2	Component 3	Component 4
	A: Potato	B: Mango	C: Avocado	D: Fibre
	g	g	g	g
1	30.0	45.0	15.0	10.0
2	30.0	15.0	45.0	10.0
3	81.0	0.0	15.0	4.0
4	62.0	0.0	30.0	8.0
5	90.0	0.0	0.0	10.0
6	24.0	33.8	33.8	8.5
7	81.0	15.0	0.0	4.0
8	62.0	30.0	0.0	8.0
9	4.0	45.0	45.0	6.0
10	51.0	22.5	22.5	4.0
11	51.0	22.5	22.5	4.0
12	46.5	33.8	11.3	8.5
13	46.5	11.3	33.8	8.5
14	62.0	30.0	0.0	8.0
15	51.0	45.0	0.0	4.0
16	94.0	0.0	0.0	6.0
17	4.0	45.0	45.0	6.0
18	51.0	22.5	22.5	4.0
19	51.0	0.0	45.0	4.0
20	62.0	0.0	30.0	8.0

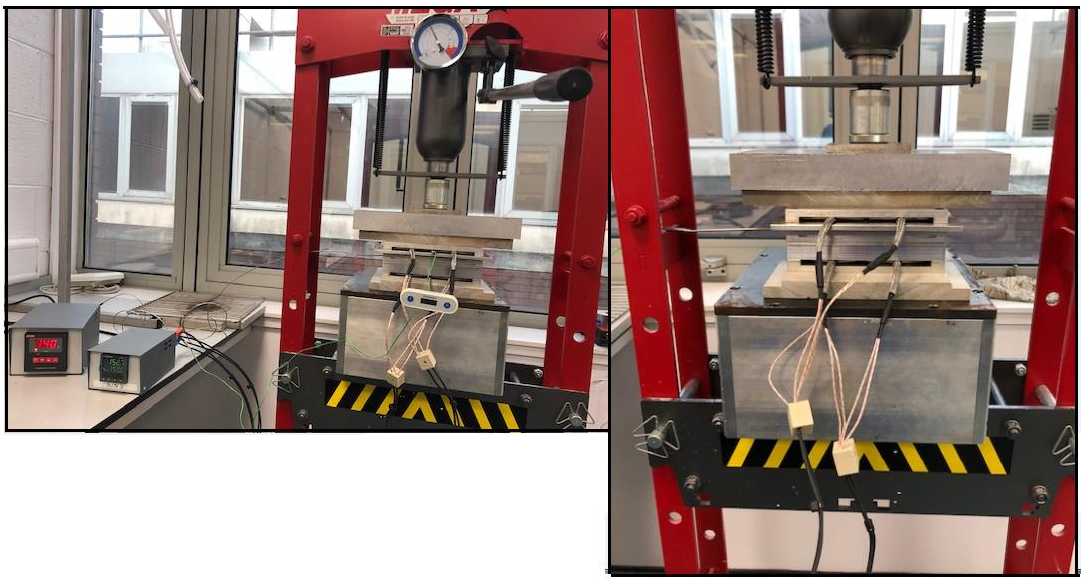


Figure 42: Thermo-compression step of the production process of the bio-plastic sheet.

Figure 43: The starch isolated from the three biomass.

b) Testing Procedures Of The Bio-Plastic Sheet Produced

In order to characterise the quality and properties of the bio-plastic sheet produced, four tests were carried out on each sheet. 3-D optical microscope is a device used widely in studying polymer characteristics, analyse the morphology structure of the bio-plastic, providing granule micrographs and showing an overview of the fracture behaviour. Therefore, it can help to determine the homogeneity, cracking, and fineness of the bio-plastic produced [99, 103]. Additionally, there are many tests that can be carried out on bio-plastic sheet to analyse its mechanical, thermal and physical properties such as; tensile strength, elongation, flexural stiffness, impact strength, degradation, water uptake, density, solubility, glass transition temperature (T_g), melting point (T_m), etc. The tests were carried out according to one of the International testing standards for bio-plastic such as; ASTM (American Society for Testing and Materials) [86, 294, 295]. However, in the present study four tests were carried on each sample, these four mechanical and physical properties in the study were the responses of this stage. For each test, three samples were cut off from each composite sheet produced according to the sample specification of each test. To minimise the error and obtain accurate results, the average of the three reading was the one considered. Table 15 below illustrates the standard test method, sample specifications, and equipment used for quality and properties tests of the bio-plastic sheet.

Table 15: The samples specifications, equipment and standard test method used in each test.

Test	Mechanical properties tests			Physical test	Quality test
	Tensile test	Flexural stiffness test	Impact strength test	Density	Homogeneity
ASTM used and Samples specifications	The specimens are cut according to ASTM D638-14 type V tensile bar (see Figure 44)	The samples are cut off according to the sample specification described in ASTM D790-03 (see Figure 44)	The samples are cut off according to the sample specification described in ASTM D6110 – 04 (see Figure 44)	The samples are cut off according to ASTM D792-08 (Vol. not less than 1cm^3, thickness at least 1mm for each 1g, weight 1-5g with smooth and surface edges).	A small specimen with any dimensions can be used
Equipment	5 kN Zwick's universal electro-mechanical testing machine model Z5	5 kN Zwick's universal electro-mechanical testing machine model Z5	Charpy impact testing machine	Pycnometer	3D optical microscope

Figure 44: Test specimens for the four tests.

The following outline and describe the four tests carried out on each sheet produced as well as the homogeneity test which was only applied on the two specimens. Appendix F illustrates the dimensions of the specimens of each test.

1. Tensile Strength

The amount of plasticiser has a major impact on the strength of the bio-plastic. There is several equipment can be used to measure the tensile strength, such as; Zwick's 5 kN universal electro-mechanical testing machine. Furthermore, several standards can be followed to implement tensile strength like, ASTM D882-12 which used for thin plastic sheeting (less than 1.0 mm in thickness) [294, 296]. ASTM D638-14 is a standard test applicable for any thickness of unreinforced and reinforced plastics up to 14 mm [103]. In the present study, the test was carried out according to ASTM D638-14. Figure 45 shows Zwick's testing machine used for testing the tensile strength of the bio-plastic sheets.

Figure 45: Zwick's testing machine.

2. Flexural Stiffness

Flexural test or as it is well-known bending test determines bending properties of a material. It measures the ability of the material to resist bending deformation under loads. ASTM D790-03 is a standard test method for flexural properties of unreinforced and reinforced plastics and electrical insulating materials [103, 297].

The procedure described in ASTM D790-03 was followed on all samples using Zwick's 5 kN universal electro-mechanical testing machine model Z5 shown in Figure 45. Based on the dimensions of the specimen and the bending test tool of the machine, the machine was set to stop the test when the sample reached 62% deflection or if the sample broke before 62%.

3. ***Impact Strength***

Charpy impact tests is a quick and simple common method used to test the impact properties of the polymers. ASTM D6110 – 04 is a standard test method was followed for determining the Charpy impact resistance of a bio-plastic sheet. This test determines the kinetic energy required to initiate fracture and continue the fracture until the specimen tested is broken [298]. Figure 46 shows the Charpy impact testing machine used.

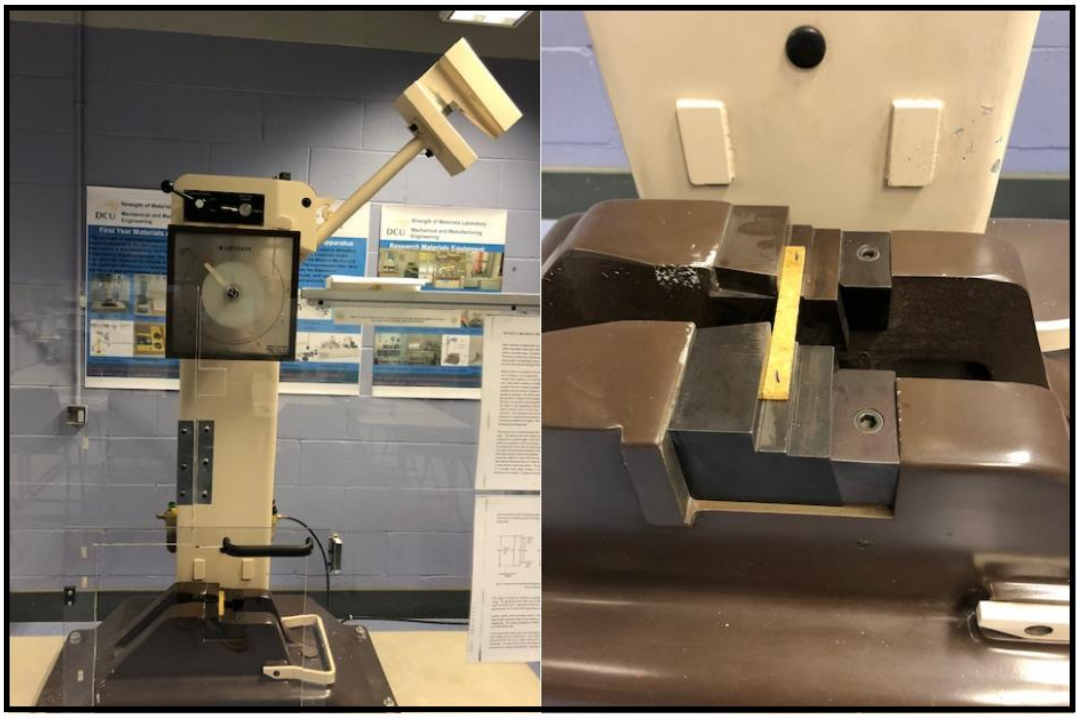


Figure 46: Charpy impact testing machine.

4. ***Density***

Density is an important property of plastic. It is very necessary, as it can greatly help in; studying the physical changes of the specimens, calculating the cost-weight ratios, etc. [299]. Density test determines the density (expressed in $kg/m^{3)}$ and specific gravity (Relative density) of a solid plastic. ASTM D792-08 was followed

to measure the density of the bio-plastic sheet produced. The test was carried out using Pycnometre device as shown in Figure 47.

Figure 47: Pycnometre device.

- ***The Homogeneity Of The Starch and Fibre***

3D light optical microscope was used in order to evaluate the homogeneity of the starch and fibre within the bio-plastic sheets produced. Two specimens were cut by the cutting machine from two different areas of two bio-plastic sheets. Figure 48 shows the microscope used.

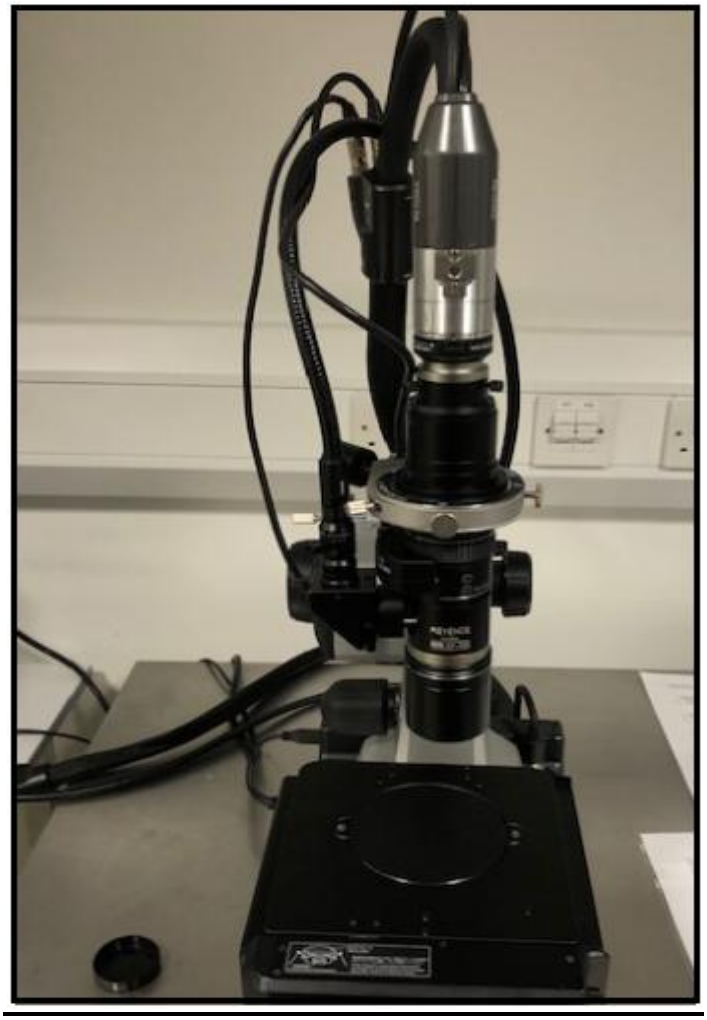


Figure 48: 3D light optical microscope.

3.6 Energy Balance And Cost Effectiveness

The energy balance and cost effectiveness were carried out in the study to evaluate the economic influence of the incorporation of the bio-plastic sheet production process on the profitability of the AD process.

3.6.1 Energy Balance Of The AD

The energy balance of the AD was carried out by comparing the energy content of the optimal biogas generated by the pre-treated biomass with the total electric energy consumed in the digestion process. Table 16 shows the equations were applied to calculate the energy gain/loss of AD of each of the three biomass separately:

Table 16: The equations used for working out the energy balance.

Equations		
*	$B_S = CH_4\ [\%] * 9.67$	Eqn. 9
*	$E_P = B_S * B_P$	Eqn. 10
*	$E_C = \frac{E_{PT}}{VS_m}$	Eqn. 11
*	$\text{Net } E_P = E_P - E_C$	Eqn. 12
*	$\text{Energy balance;} = \frac{E_P - E_C}{E_C} * 100$	Eqn. 13

Definition of each component	
B_S	is the energy content of the biogas produced by biomass [kWh m^{-3}].
CH_4	is the average of methane content [%].
9.67	is the energy content of 1 Nm^3 of biogas [kWh/Nm^3]
B_P	is the quantity of biogas produced for each gram of VS of the biomass [$m^3 g^{-1}$ VS].
E_P	is the energy of the biogas produced from 1 g of VS of the biomass [kWh g^{-1}VS].
E_{PT}	is the electric energy consumed during the digestion measured by a kilowatt hour meter [kWh].
VS_m	is the total quantity of VS into the flask [g].
E_C	is the electric energy consumed during the digestion to process 1 g of VS of biomass [kWh g^{-1}VS].
Net E_P	is the total energy produced by 1 g of VS of biomass pre-treated.
Energy gain / loss	is the difference in percentage between the energy provided by the biogas produced from biomass (E_P) and the total electric energy consumed in the process. If the (E_C) is > than the (E_P), that's means the AD of the biomass is not economically feasible.

3.6.2 Cost Effectiveness Of The Bio-Plastic Sheets

The cost effectiveness of the manufacturing process of the bio-plastic sheet was carried out by comparing the operating cost of the production of the bio-plastic sheet (including the energy consumed and the cost of the materials used) and the hourly labour wages in the Republic of Ireland with the value of the bio-plastic sheets produced. The electric energy consumed in the chemical pre-treatment of mango coats was considered in the calculation of the cost effectiveness of the manufacturing process. The value of the bio-plastic sheet was calculated based on its density. According to the Wageningen food and bio-based research [300], the prices of a starch blend bio-plastic that has a density of 1250 –1350 kg/m^3 are in the range of (2 to 4 euro/kg).

3.7 Chapter Summary

The uses of the Hollander beater were not limited in the laboratory for pre-treating the biomass. The beater was also employed to separate the starch and prepare mango seed coats. For isolating the starch from the seeds of the mango and avocado, the seeds were processed separately (before they were mixed with the peels and the other residues). This was done in order to facilitate the separation process, obtaining the largest possible starch amounts and therefore, making the AD feedstocks as much as possible free of starch. However, the integration of the implementation of these processes into a single equipment (the Hollander beater) has led to the realisation of the concept of bio-refinery, which involves the integration of equipment for the production of bio-materials, bio-energy, and so on.

Another main concept of bio-refinery was also achieved by alternative uses of the bio-wastes. In order to meet the concept of bio-refinery that "make full use of the biomass" and for benefiting from as much waste as possible and thus increase the contribution of AD in waste management, the total waste of the three biomass were exploited. Additionally, all materials used in the experiments were extracted from the three biomass except glycerine.

The next chapter reports the results obtained from each planned experiment along with a discussion of the main findings.

Chapter 4 : RESULTS AND DISCUSSION

4.1 Introduction

This chapter is divided into two main parts, further divided into sections and sub-sections. Both parts describe and analyse the results have been obtained from the AD processes of the three biomass and the results of the production process of the bio-plastic sheet. The chapter also discusses the laboratory results of the digestate and the major findings from the experimental work. The experiments were designed and analysed using two techniques: BBD and mixture design technique. The yield and quality of the biogas produced from each run, are based on five responses of the AD process, while, the four properties tested are the responses of the bio-plastic sheet production process. Figure 49 illustrates the structure of this chapter.

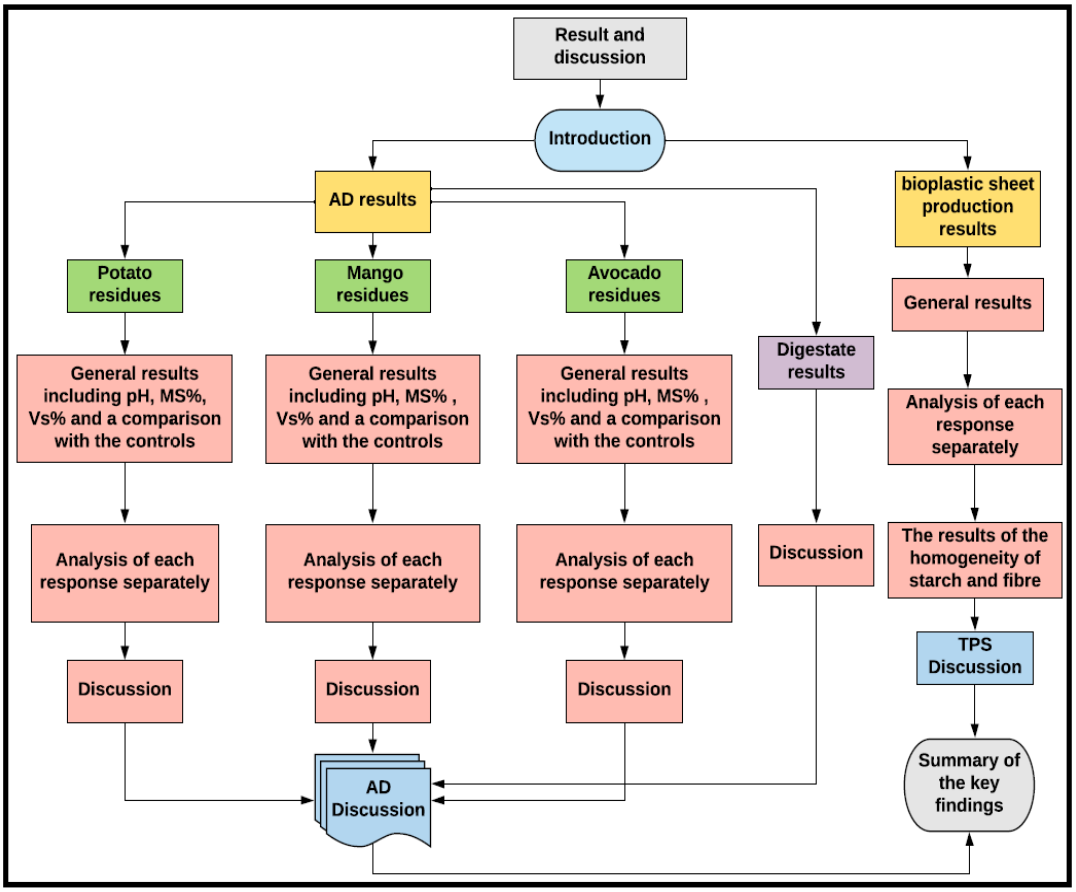


Figure 49: The structure of Chapter 4.

4.2 Anaerobic Digestion Results

The AD process of each biomass was carried out according to the design matrix illustrated in Table 13. For safety purposes, i.e. to avoid the leakage of the gas, the gases were collected twice on day 7 and on day 21. During the first collection, only the biogas yield were measured and recorded. While, on the second collection the biogas yield, pH level, the methane concentration and carbon dioxide concentration were measured and recorded. The two biogas yields were added to each other to find the total biogas volume. As aforementioned, the organic concentration (g-VS) and sludge concentration (%) levels of all biomass were adjusted by dilution with water to 1.6, 4.05 and 6.5g and 20, 35 and 50% respectively. Furthermore, the water baths were connected to the electricity via energy consumptions meters to measure the electric energy consumed, to calculate the energy balance. The following sub-sections depict the results obtained from each biomass.

4.2.1 AD Of Potato Residues

The potatoes were washed thoroughly to remove any impurities and peeled. Approximately, 125g peels were extracted from each 1 kg of potatoes. The starch isolated constituted 14±2% of the total weight of the peels. The peels, were directly processed in the beater and fed into the digester that after isolating the starch and adjusting the organic concentration (g-VS) according to the design matrix. Prior to discharging the pre-treated biomass from the beater, the biomass mixture was stirred, and a pre-determined quantity was sampled. The organic concentration (g-VS) of the sample were adjusted to 6.5 g-VS. After stirring, nine samples were poured into the reactors and blended with 50% sludge. Each three reactors were distributed in a water bath at one of the three temperature levels. These nine reactors were used as controls to evaluate the impacts of the starch on the responses.

Appendix G present the TS%, MS% and the adjusted weights of the VS and TS at each VS level of the pre-treated biomass before and after the separation of starch from each biomass. Additionally, Figure 50 and Figure 51 graphically show the TS and VS before and after separation of the starch from the potato residue. Figure 52, Figure 53, Figure 54 and Figure 55 graphically show the results of all responses including the pH levels after digestion of each sample (also see Appendix H). Table 17 shows the comparison between the results obtained from the controls with the

predicted results of the potato residues after separation of starch. Notably, the predicted results were generated by the DOE at the same conditions of the controls (6.5 g-VS and 50% sludge at the three temperatures).

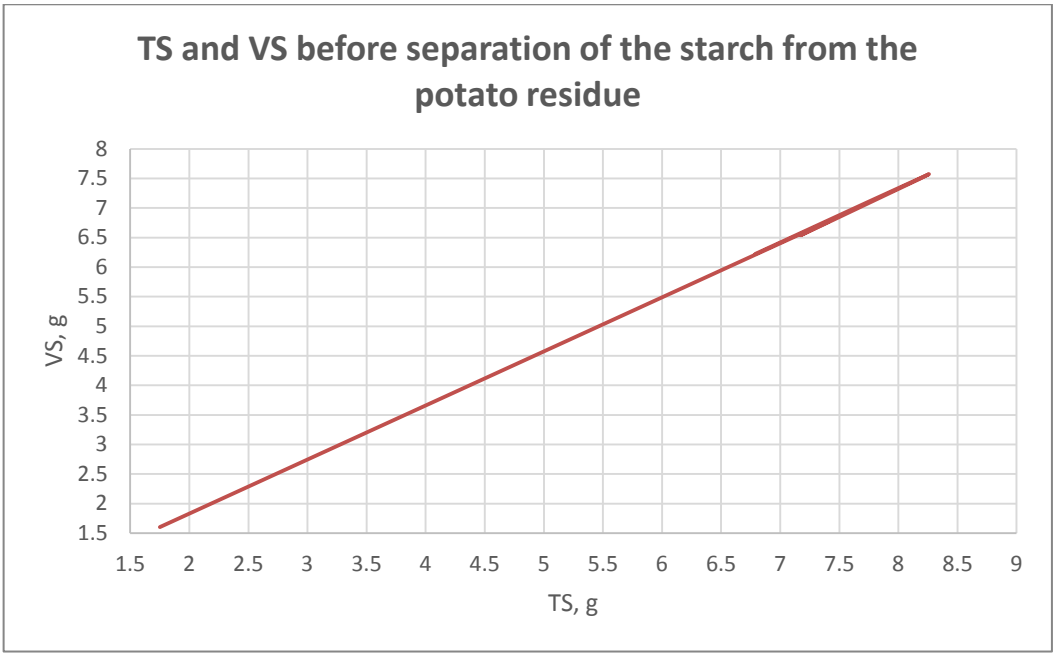


Figure 50: TS and VS before separation of the starch from the potato residue.

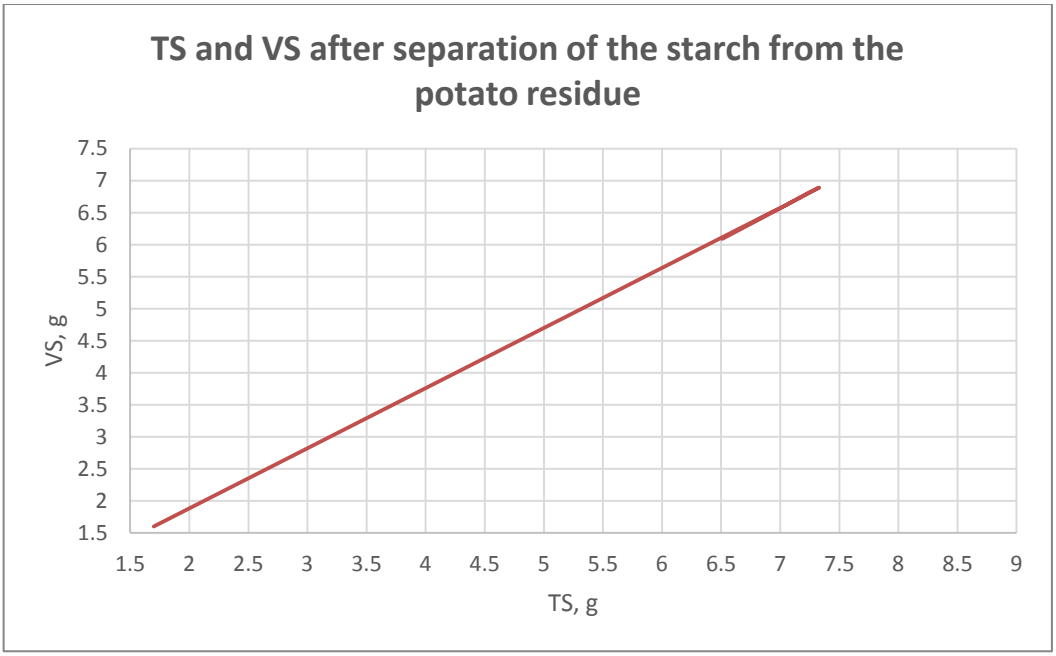


Figure 51: TS and VS after separation of the starch from the potato residue.

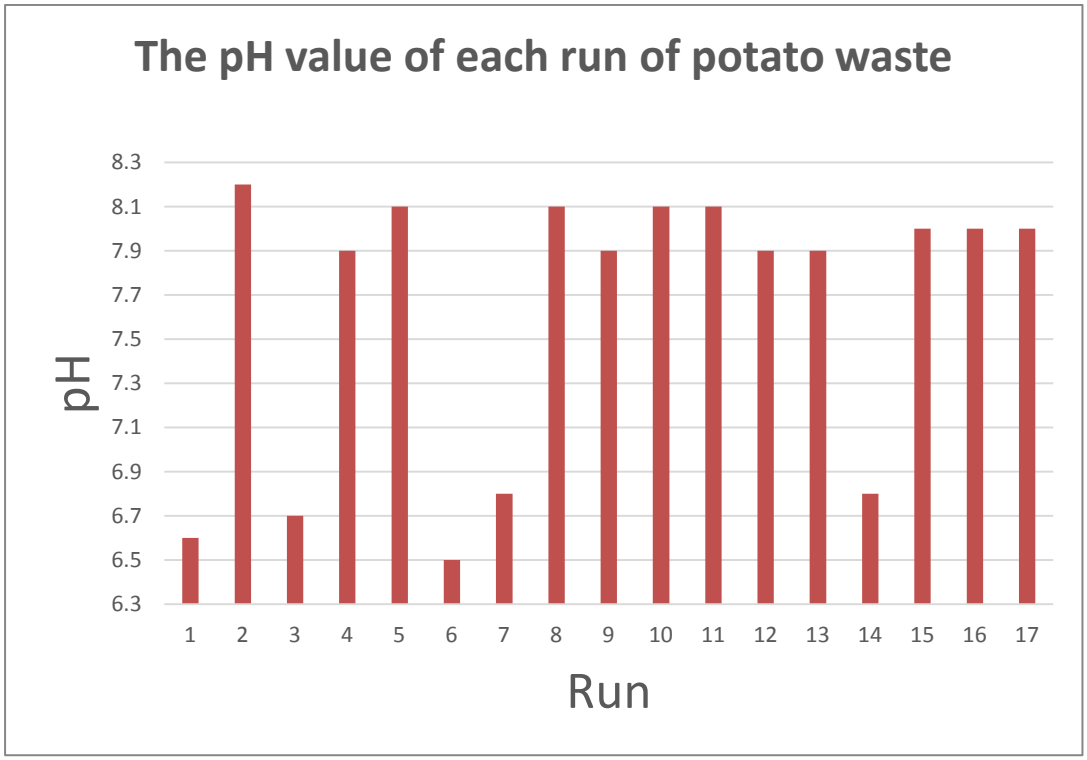


Figure 52: The pH values of each run of potato waste.

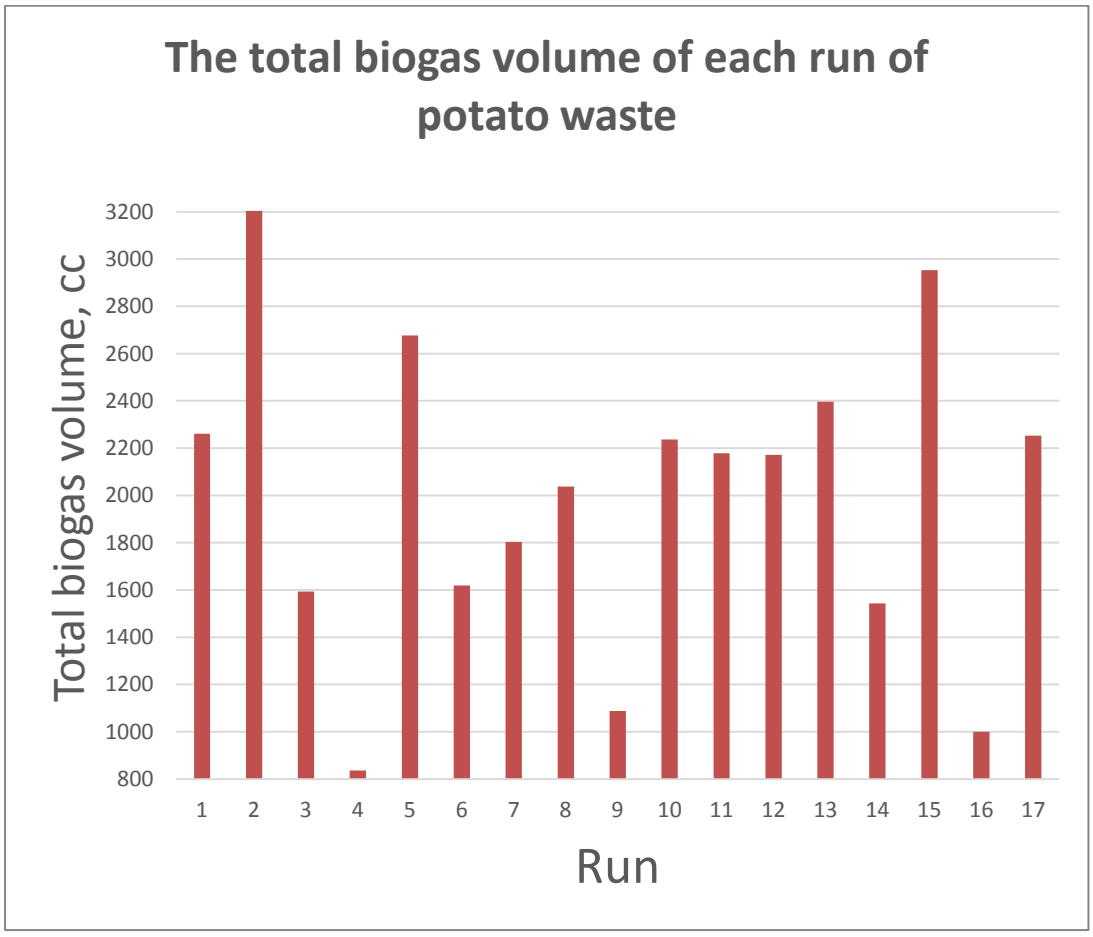


Figure 53: The total biogas volume of each runs of potato waste.

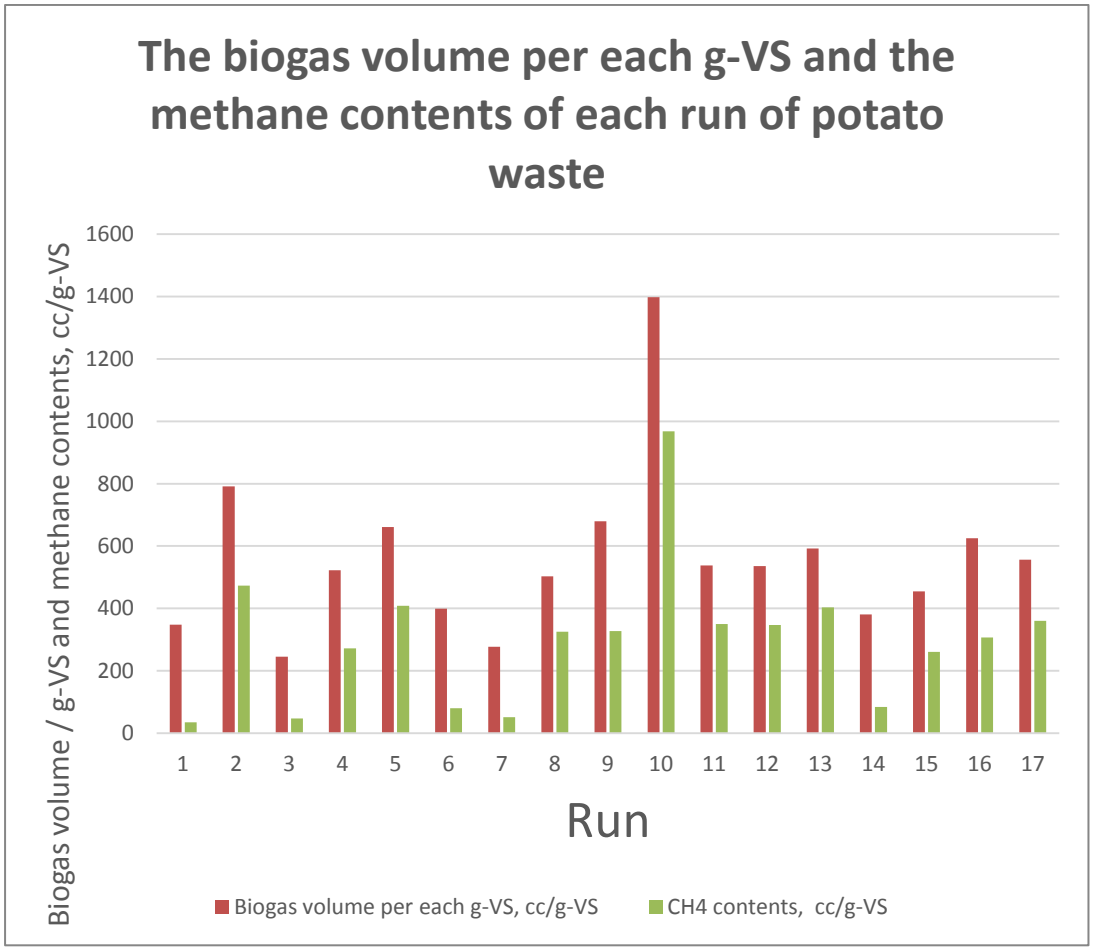


Figure 54: The biogas volume per each g-VS and the methane contents of each run of potato waste.

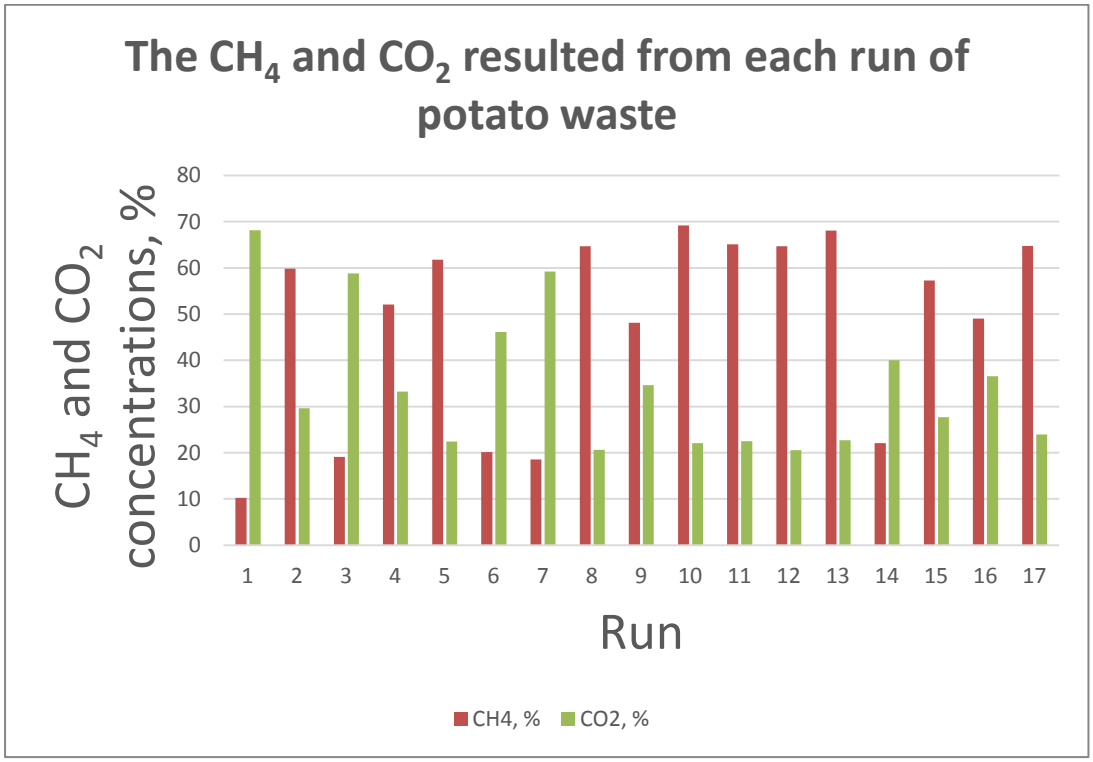


Figure 55: The CH_4 and CO_2 resulted from each run of potato waste.

Table 17: A comparison between the results of the controls and the predicted results at same conditions.

	Sample no.	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4 %	CO_2 %	CH_4 content /g-VS
Actual (controls)	1	32	6.5	50	7.9	2448.0	376.6	40.5	38.4	152.5
	2	35			8	3101.1	477.1	57.9	29.9	276.2
	3	38			7.9	2855.0	439.2	38.8	42.5	170.4
Predicted	1	32	6.5	50		2627.3	350.8	39.9	38.8	144.3
	2	35				3136.2	481.9	57.0	30.2	320.3
	3	38				2871.9	400.7	38.0	43.1	169.4
Difference %						-7.3	6.9	1.5	-1.1	5.4
						-1.1	-1.0	1.6	-1.1	-15.9
						-0.6	8.8	2.0	-1.4	0.6

In order to simplify the comparison between the biogas produced from each biomass, the ranges of the three factors were adjusted to be the same (unified). A number of preliminary trials were done to adjust the range of each of the three factors. The preliminary trials of setting the range of the organic concentration revealed that, the excessive feeding of the digester cause failure to the system, while the insufficient feeding lead to a starving condition therefore, the organic concentration range was set to be 1.6 to 6.5 g-VS.

From Appendix G, it can be noted that the average dry matter weights (TS) of the biomass before and after the separation of starch from potato residues (before adjusting the VS weight to the required organic concentration level) were approximately 7.42 and 7.03g respectively. Based on the two average TS weights (the primary TS weights), the TS weights at the required organic concentration level were calculated by applying Equation 41 in Appendix D (also see Figure 50). For instance, if the resulted average dry matter (the primary TS weight) was 10g and the VS weight was found to be 9 g-VS, the dry matter weight at 6.5 g-VS would be = $\frac{10*6.5}{9} = 7.22$g and therefore, the ashes= 7.22-6.5= 0.72g. Determining the primary TS weights helped in calculating the dry matter weight at each organic

concentration level and thus, adjusting the organic concentration to the required level using water dilution.

In order to calculate the biogas volume for one gram VS of each sample had organic concentration of 6.5g-VS, the total volume produced was divided by the organic weight (6.5g). In contrast, the total volumes of the biogas were divided by 4.05g and 1.6g for each sample had the middle and lowest organic concentration levels respectively.

As shown in Figure 52 and Appendix H, the pH levels of all samples ranged from 6.5 to 8.2. This indicates that the systems and digesters were in equilibrium and stable. It was also observed that there is a significant influence of the interaction between the organic and sludge concentrations on the pH levels. When the organic concentration at the higher concentration and the sludge at lower concentration the pH level decreases and vice versa.

During the second collection of the biogas, a drop in the volumes of the biogas produced was noticed in almost all of the samples. Figure 53 shows that, the highest total volume of the biogas resulted was approximately 3204 cc at 38 ^{0}C, 4.05 g-VS and 50% sludge. While, the lowest was 836 cc at the condition of 32 ^{0}C, 1.6 g-VS and 35% sludge. In terms of the biogas volume produced by 1 g-VS from each sample, it is clear that the highest amount of biogas produced was 1397.9 cc/g-VS for run 10 and the lowest was 245 cc/g-VS for run 3 (see Figure 54 and Appendix H). Figure 55 also reveals that Run 10 has recorded the highest methane concentration comparing to the other samples where, the methane concentration was constituted about 69.2% (967.4 cc/g-VS) of the total gas produced by each gram VS. It is clear that, the organic concentration and sludge concentration have major influences on the methane concentration and carbon dioxide concentration.

In reference to Table 17, it can be noted that the differences between the results of the three controls and the predicted results were relatively low, especially the differences relating to the quality of the biogas. This can be due to the low difference of the weights between the control reactors and the others, as the starch weight was the only difference between the two of them.

1. Analysis Of The Responses

This section provides the reader with an analysis of the results obtained from each response. The analysis was carried out through the aid of the DOE, to identify the significant factors (parameters) and their interactions influencing the response. ANOVA was used in all responses to test the adequacy of the models developed and to identify the influence of each factor and the interactions of factors on the response. In the ANOVA, if the P-value of the model and of any term does not exceed the level of significance (α= 0.05), they are considered statistically significant within the confidence interval of (1- α). In terms of the lack-of-fit test, it is considered insignificant if the P-value exceeds the level of significance. The values of R^2, adjusted R^2, and predicted-R^2 are other tools to further examine the model. Where, the values of these tools are closer to 1, it indicates that the regression of the model is good. The next five sub-headings describe the analysis of each response.

a. <u>Total Volume Of Biogas Produced</u>

Table 18 is the ANOVA analysis of this response. It is obvious that the developed model was significant as well as the model terms B, C, A^2, B^2 and C^2. Additionally, the P-value related to the 'Lack of Fit', was > 0.05. Thus, the Lack of Fit was not significant. Therefore, the model was adequately fit the data. The ANOVA found that the "Pred R-squared" and " Adj. R-Squared" for the model were in reasonable agreement. The "Adeq Precision" was found to be greater than 4. These indicated that the model was adequately fit the data and therefore, can be used to navigate the design space. Equation 14 shows the final equations in terms of coded and Equation 15 shows the mathematical model in actual values. From Equation 14, it can be seen that the sludge concentration term (C) has the most significant influence on the total biogas volume (increase the response). The analysis is showing that, there was no significant influences of any interactions of the parameters on the total biogas volume.

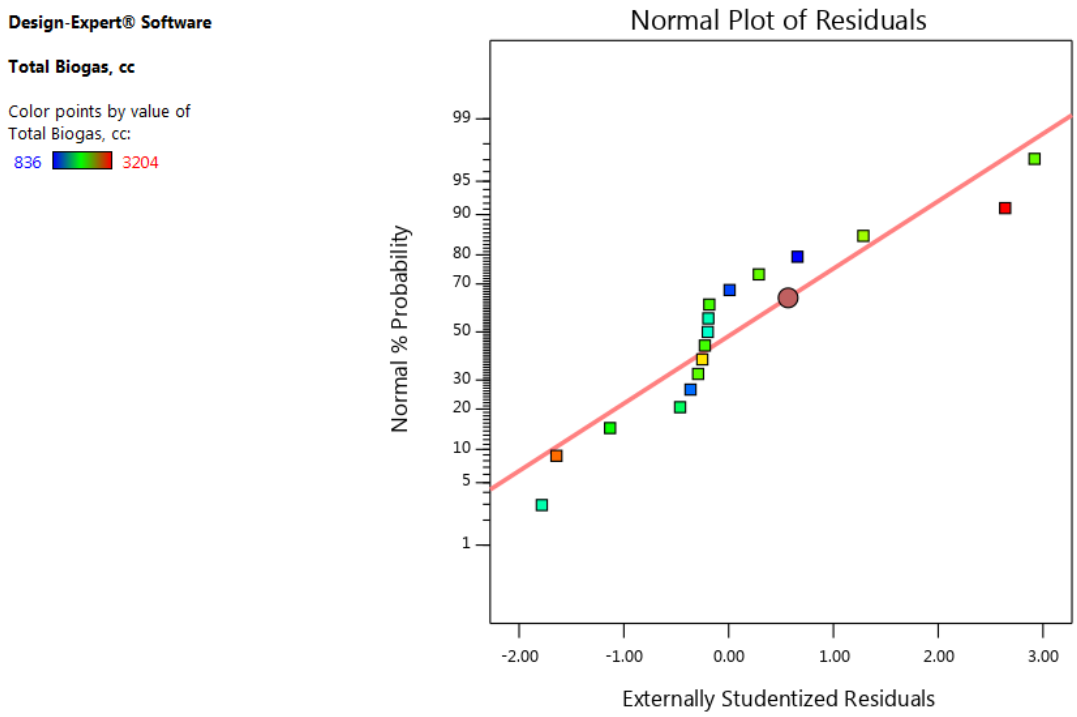
Table 18: The ANOVA table for the total volume of biogas produced response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	6680425.9	6	1113404.3	38.565	0.000002	significant
A-Temperature, °C	119637.94	1	119637.94	4.143901	0.069148	
B-Organic Conc., g-VS	1488649.2	1	1488649.2	51.56236	0.000030	
C-Sludge Conc., %	2598310.9	1	2598310.9	89.99772	0.000003	
A^2	629261.36	1	629261.36	21.79573	0.000883	
B^2	1106606.3	1	1106606.3	38.32953	0.000103	
C^2	815974.8	1	815974.8	28.26293	0.000340	
Residual	288708.52	10	28870.852			
Lack of Fit	219822.52	6	36637.086	2.127404	0.242636	not significant
Pure Error	68886.002	4	17221.501			
Cor Total	6969134.4	16				
R^2 = 0.96				Adj R^2 = 0.93		
Pred R^2 = 0.86				Adeq Precision = 21.84		

$$\text{Total Biogas, cc} = 2207.333 + 122.2896 * A + 431.3713 * B + 569.9025 * C - 386.587 * A^2 - 512.659 * B^2 + 440.2204 * C^2 \quad \text{Eqn. 14}$$

$$\text{Total biogas, cc} = -52885.2 + 3047.552 * (\text{temperature, °C}) + 867.871 * (\text{organic concentration, g} - \text{VS}) - 98.964 * (\text{sludge, \%}) - 42.9541 * (\text{temperature °C})2 - 85.4075 * (\text{organic concentration, g–VS})2\ 1.956535 * (\text{sludge, \%})^2 \quad \text{Eqn. 15}$$

Figure 56* and Figure 57 are residual normality plot and the predicted values versus the actual values plot respectively. They are contributing in validating the strength of the developed model and illustrating the correlation between the measured and predicted response values. Figure 56 is indicating that the residuals are normally distributed (The normal distribution is always symmetrical about the mean). The distribution of most of the points in Figure 57 on the diagonal line or closer to it means that, the model has predicted the results very well and therefore, there was a good correlation between the model's predicted results and the actual results.



∗ Label uses US English due to Software - Hence 'Color' = 'Colour'.

Figure 56: The normal plot of residuals.

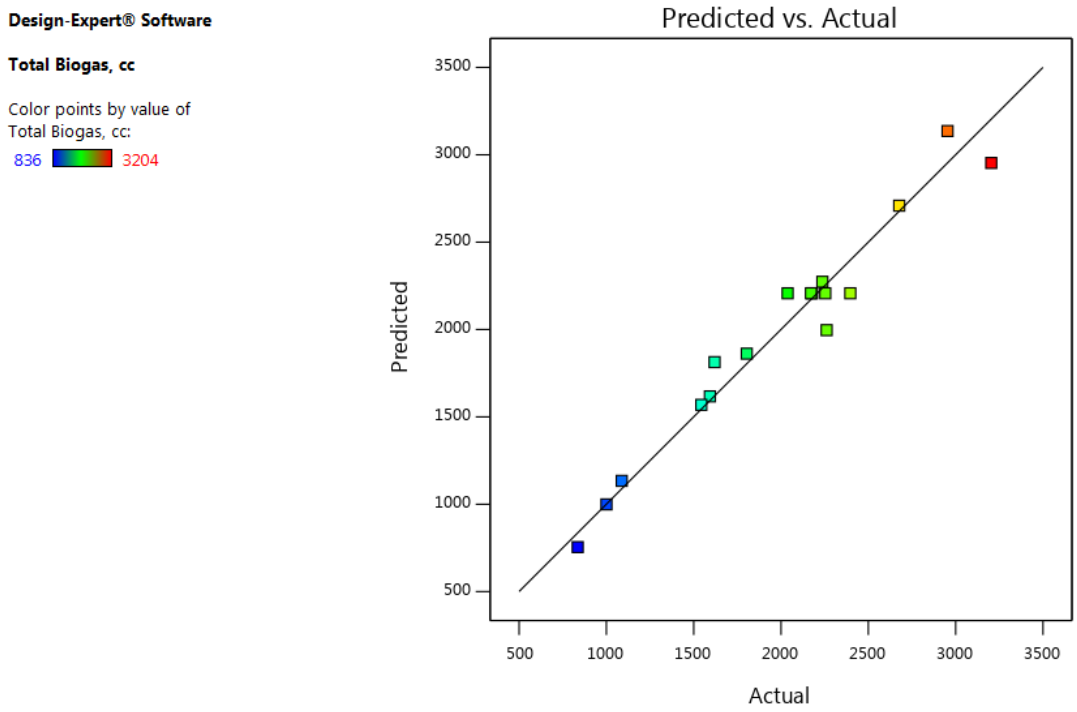


Figure 57: The scatter plot of the predicted values versus the actual values.

The perturbation plot shows the behaviour of each factor and its influence on the response. Figure 58 shows the behaviour of the three factors and their influences on the total biogas volume. In this figure, the deviation from the reference point (35

^{0}C, 4.05 g-VS and 35% sludge) is represented as coded values. It is evident that the total biogas volume decreases when the temperature deviates either to higher or lower temperature than approximately 35° C (reference point). Looking at Figure 58, an increasing pattern can be observed in the total biogas volume when the sludge concentration is increased. In other words, there was a direct proportional between the sludge concentration and the total biogas volume, when the sludge concentration was increased, the total biogas volume increased and vice versa. Additionally, a less increasing pattern can be also observed in the total biogas volume when the organic concentration (g-VS) increased from the lowest level. When the organic concentration reached a little bit higher than the reference point, the total volume starts decreased.

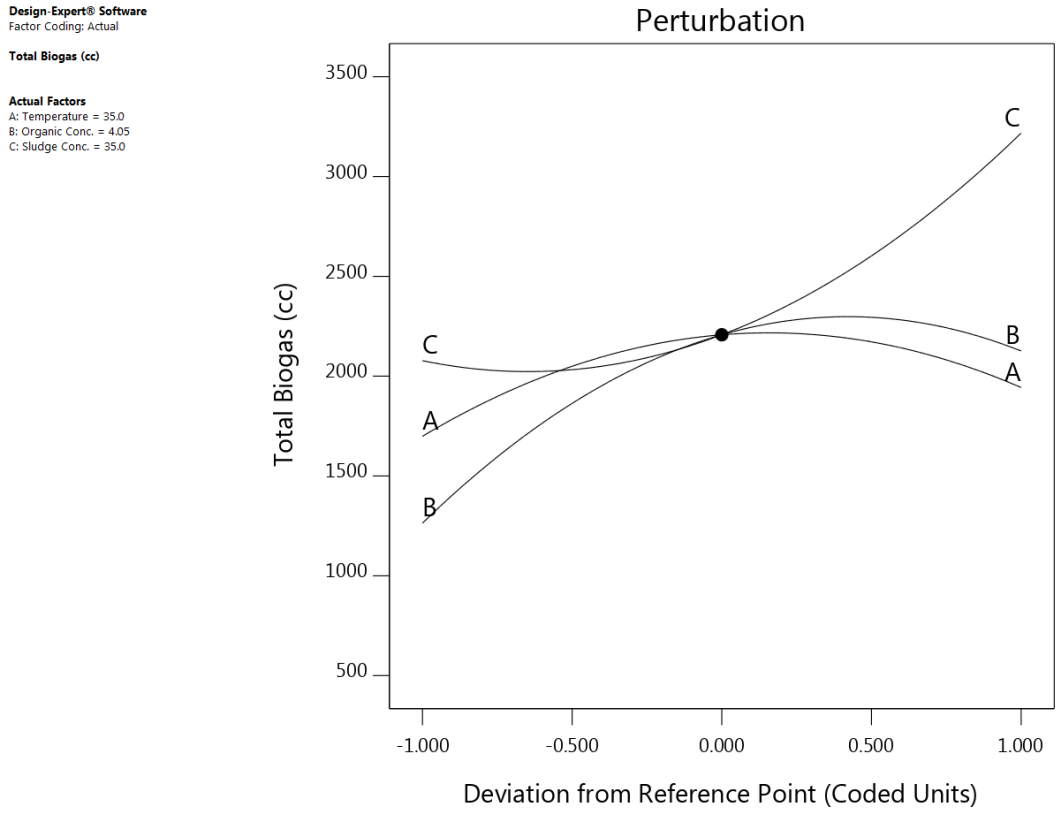


Figure 58: The Perturbation plot of the total volume of biogas.

b. <u>The Volume Of Biogas Produced From Each Gram VS</u>

This response is the volume of the biogas produced from each gram VS in every samples. Table 19 shows the ANOVA and Equation 16 illustrates the mathematical model equation for the response. As is clear from the equation, the mathematical model of this response was transformed to natural log as by the

recommendation of the software. Table 19 shows that the model was significant. The model terms A, B, C, BC, A^2, B^2 and C^2 have significant influences. Moreover, the "Lack of Fit" was not significant. Hence, the model adequately fit the data. In terms of the R^2, adjusted R^2, and the predicted-R^2, they were all close to 1, therefore, the regression of the model was good. The "Adeq Precision" confirmed the fitness of the data. It was also shown that the organic concentration (B term) has the most significant influence. In addition, the sludge concentration (C term) as well as the interaction of (BC) term have significant influences. Moreover, Equation 17 illustrates the final equation in terms of coded factors as determined by ANOVA for biogas volume produced from each VS. This equation confirms that organic concentration (B term) has the most significant influence.

Table 19: The ANOVA table for the total volume of biogas produced from each g-VS response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	2.61	7	0.373	99.6334	0.00000008	Significant
A-Temperature, °C	0.04	1	0.035	9.4328	0.01332621	
B-Organic Conc., g-VS	1.41	1	1.414	377.3640	0.00000001	
C-Sludge Conc., %	0.62	1	0.617	164.5802	0.00000044	
BC	0.05	1	0.051	13.7353	0.00487415	
A^2	0.27	1	0.265	70.8564	0.00001471	
B^2	0.04	1	0.036	9.6157	0.01270350	
C^2	0.22	1	0.217	57.9150	0.00003292	
Residual	0.03	9	0.004			
Lack of Fit	0.02	5	0.004	1.1225	0.46852398	not significant
Pure Error	0.01	4	0.004			
Cor Total	2.65	16				
$R^2 = 0.99$				Adj $R^2 = 0.98$		
Pred $R^2 = 0.93$				Adeq Precision = 42.31		

$$\text{Ln}\left(\text{Biogas volume per 1g of VS}, \frac{\text{cc}}{\text{gVS}}\right) =$$
$$-28.0571 + 1.974947 * (\text{Temperature}) + 0.061253 * (\text{Organic concentration}) - 0.03961 * (\text{Sludge concentration}) - 0.00309 * (\text{Organic concentration}) * (\text{Sludge concentrationC}) - 0.0279 * (\text{Temperature})^2 - 0.01541 * (\text{Organic concentration})^2 + 0.001009 * (\text{Sludge concentration})^2 \quad \text{Eqn. 16}$$

$$\text{Ln}\left(\text{Biogas volume per 1g of VS}, \frac{\text{cc}}{\text{g}} - \text{VS}\right) = 6.30 + 0.0665 * \text{A} - 0.4204 * \text{B} + 0.2776 * \text{C} - 0.1134 * \text{B} * \text{C} - 0.2511 * A^2 - 0.0925 * B^2 + 0.2270 * C^2 \quad \text{Eqn. 17}$$

The normal plot of residuals (Figure 59) shows that almost all points are located on the line or close to it, indicating that the residuals are normally distributed. Externally studentized residuals (i.e. student-t-test not chi-square test) were used to avoid any outliers. Additionally, the predicted values versus the actual values plot (Figure 60) illustrates that there was a good correlation between the predicted and the actual results of the model.

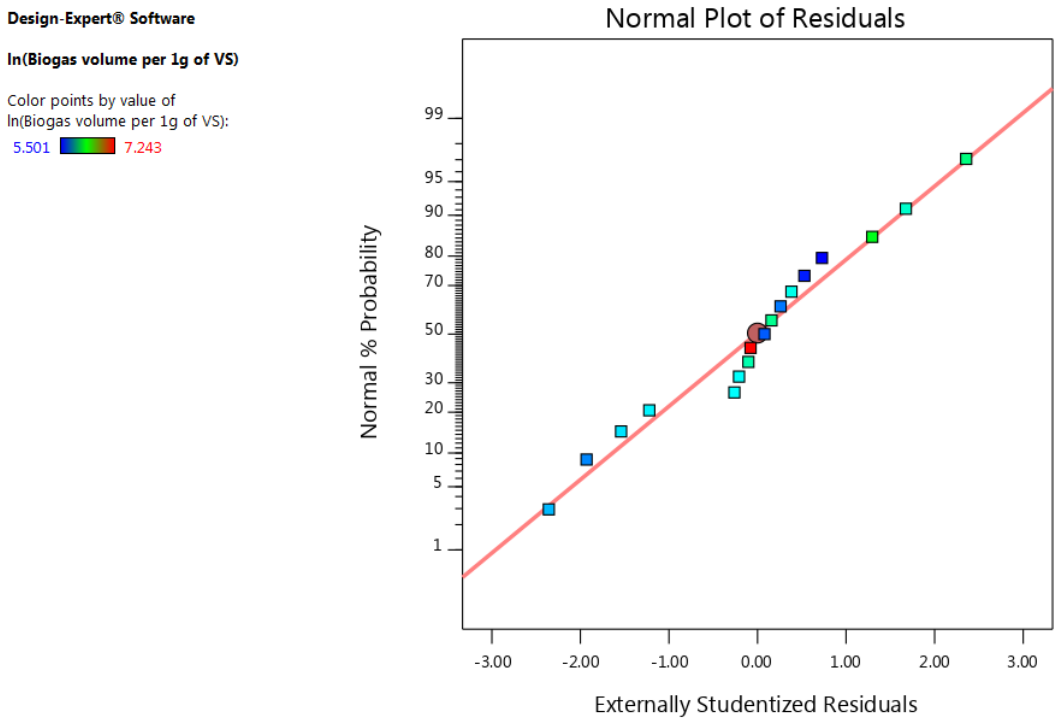


Figure 59: The normal plot of residuals.

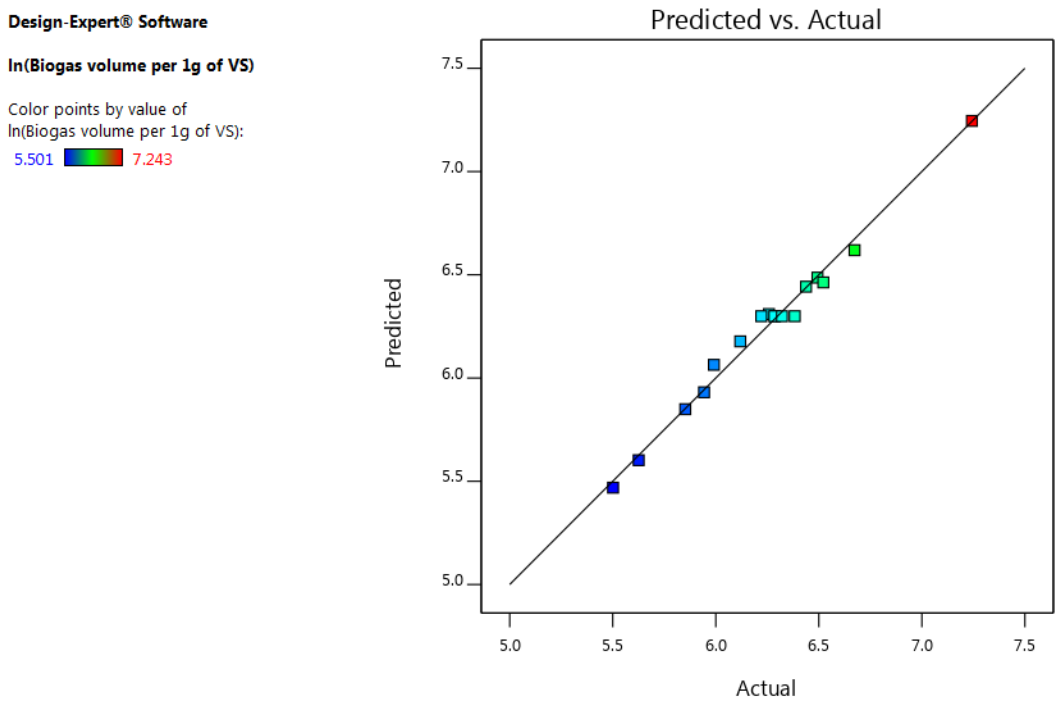


Figure 60: A scatter plot of the predicted values versus the actual values.

As shown in Figure 61, there was an inverse proportional relationship between the organic concentration and this response. This means that, when the organic concentration (B) increased, the volume decreased and vice versa. The volume of biogas from one g-VS was almost constant when the sludge concentration (C) was lower than the centre level and increased after it reached the centre level. In terms of the impact of the temperature (A) on the biogas volume produced from each gram VS, it was almost similar to its impact on the total volume of the biogas produced.

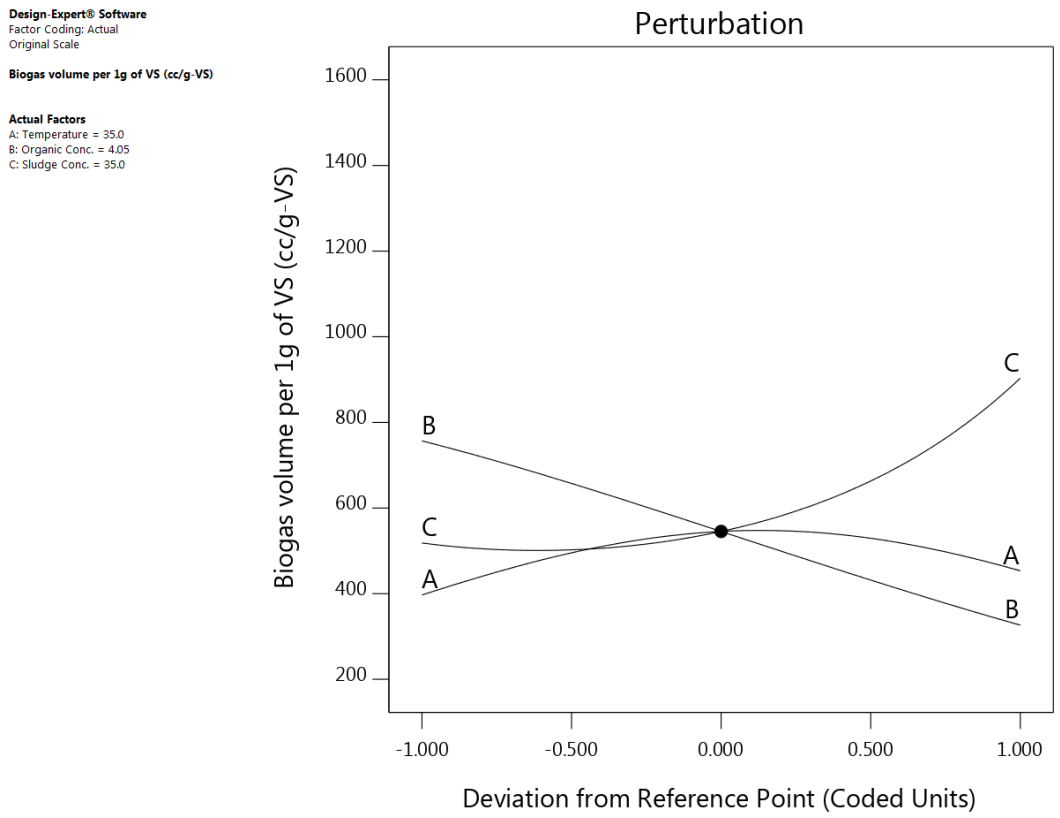


Figure 61: The Perturbation plot of the volume of biogas produced from each g-VS.

The interaction of the organic concentration (g-VS) and the sludge concentration (%) was the only interaction has a significant influence on this response. Figure 62 and Figure 63 show the influence of the interaction on the biogas volume produced from each gram VS in an interaction plot and contour plot respectively. Contour plots are three-dimensional surface plots that provide a two-dimensional view. In these plots, the contour lines are the values of the responses. All points located on the same line have the same value of the biogas volume produced from each gram VS. Figure 62 revealed that the influence of the interaction was slightly significant when the organic concentration was at its highest level. While, the biogas volume/g-VS increased as the organic concentration (B) decreased. At the lowest organic level, there was a sharp increase when the sludge concentration (C) increased. Therefore, a balance must be achieved between the concentration of the substrates and the sludge concentration. This is since the excessive feeding of reactors, as well as the lower concentration of the sludge (sludge concentration < organic concentration), negatively affect the value of the pH and thus cause a significant deficiency in the concentration of the resulting methane. In addition, the excessive feeding of the reactors caused the feedstock to not be fully

digested due to the imbalance of the bacteria population. Figure 64 also shows the influence of the organic concentration and sludge concentration in a 3D surface plot.

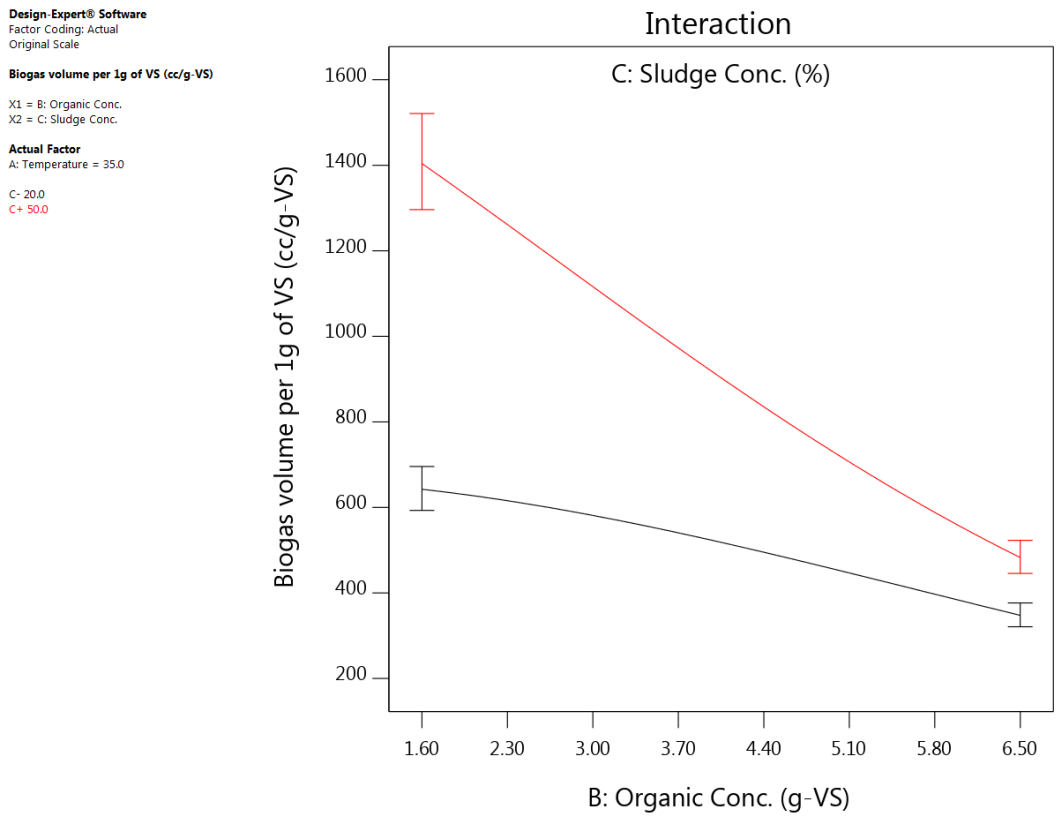


Figure 62: An interaction plot shows the influence of the organic concentration and sludge concentration on the biogas volume produced from each gram of volatile solids.

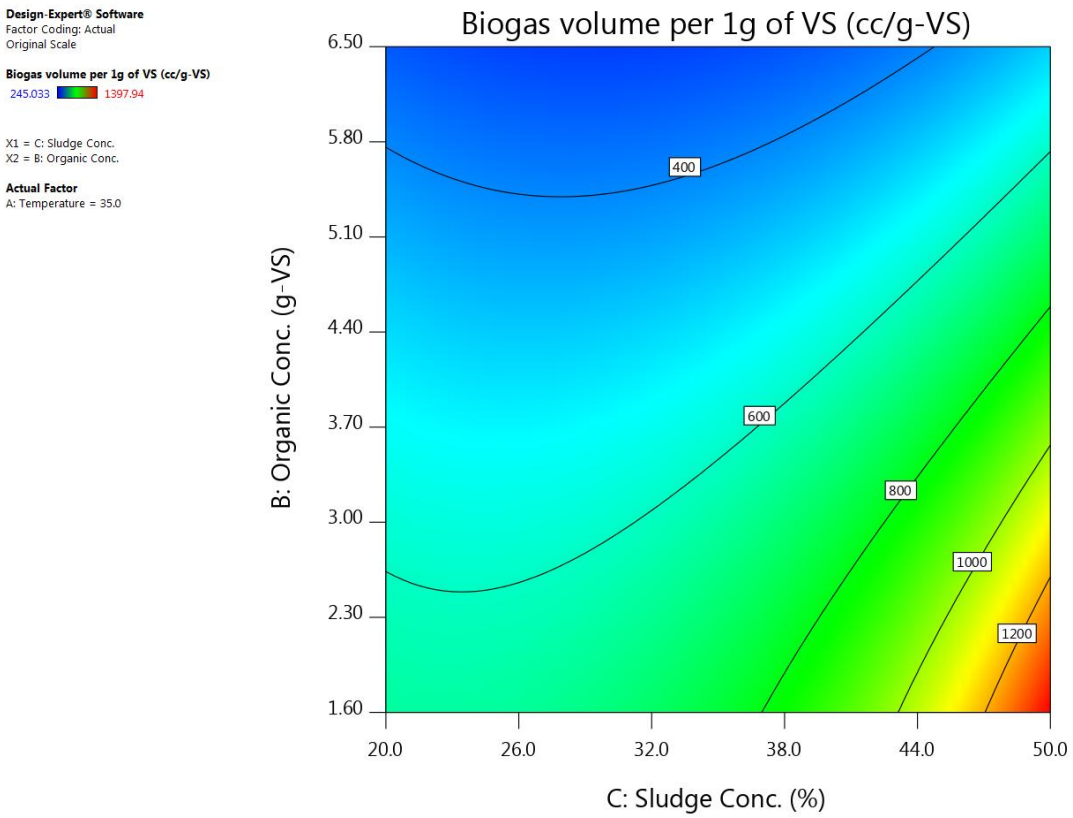


Figure 63: A contour plot shows the influence of the organic concentration and sludge concentration interaction on the biogas volume produced/g-VS.

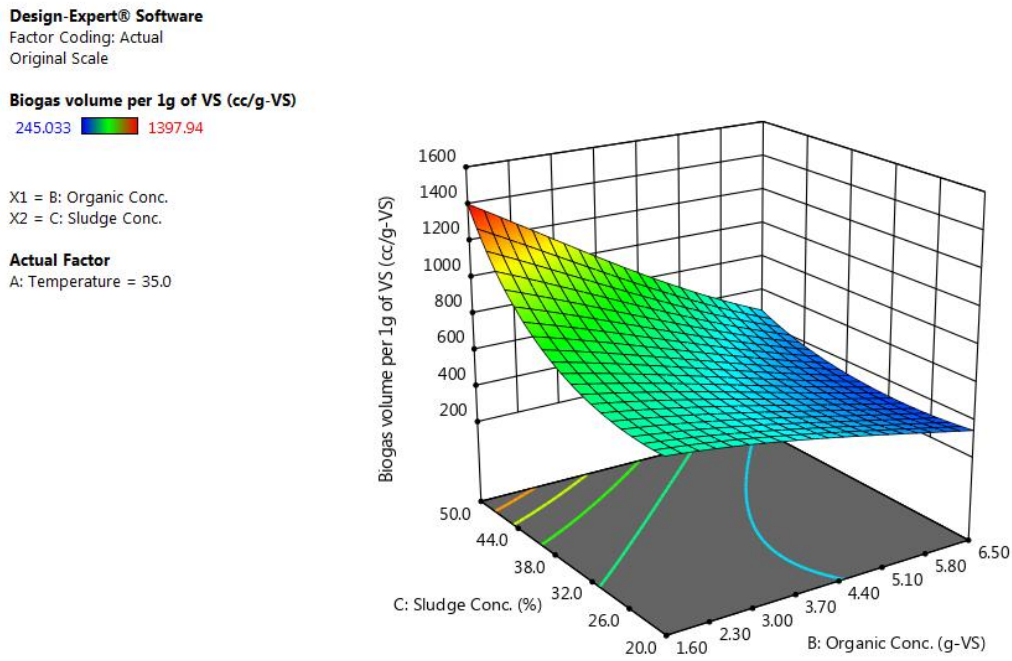


Figure 64: A 3D surface plot shows the influence of the organic concentration and sludge concentration interaction on the biogas volume produced/g-VS.

c. Methane Concentration

The ANOVA of the methane concentration response (Table 20) revealed that the model was significant and confirmed that the following terms have significant influences: B, C, BC, A^2, B^2 and C^2. On the other hand, the "Lack of Fit" was insignificant, therefore the regression of the model was good, and the model adequately fit the data. In addition, the ANOVA also confirmed that the Pred. R^2 was in reasonable agreement with the Adj. R^2. It is clear that, the sludge concentration (C term) has the most significant influences following by organic concentration (B term) (Equation 18). Also, a significant influence of their interaction (BC) can be noticed. Additionally, Figure 65 illustrates the predicted values versus the actual values plot. The distribution of most of the points in Figure 65 on the diagonal line, (i.e. close to it) means that, the model predicted the results very well and therefore, there was a good correlation between the model's predicted results and the actual results.

Table 20: The ANOVA table for methane concentration response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	6949.87	7	992.839	181.5931	0.000000006	significant
A-Temperature, °C	7.16	1	7.157	1.3090	0.28209207	
B-Organic Conc., g-VS	1603.67	1	1603.66	293.3153	0.00000003	
C-Sludge Conc., %	2721.99	1	2721.99	497.8598	0.00000000	
BC	169.00	1	169.000	30.9106	0.00035199	
A^2	1367.88	1	1367.87	250.1885	0.00000007	
B^2	686.17	1	686.175	125.5033	0.00000138	
C^2	178.31	1	178.306	32.6126	0.00029033	
Residual	49.21	9	5.467			
Lack of Fit	40.47	5	8.094	3.7070	0.11420982	not significant
Pure Error	8.73	4	2.184			
Cor Total	6999.08	16				
R^2 = 0.99				Adj R^2 = 0.99		
Pred R^2 = 0.94				Adeq Precision = 40.65		

$$\text{Methane concentration, \%} = -2441.66 + 139.8727 * (\text{Temperature}) + 5.257309 * (\text{Organic concentration}) + 2.537951 * (\text{Sludge concentration}) + 0.176871 * (\text{Organic concentration}) * (\text{Sludge concentration}) - 2.00269 * (\text{Temperature})^2 - 2.12675 * (\text{Organic concentration})^2 - 0.02892 * (\text{Sludge concentration})^2 \quad \text{Eqn. 18}$$

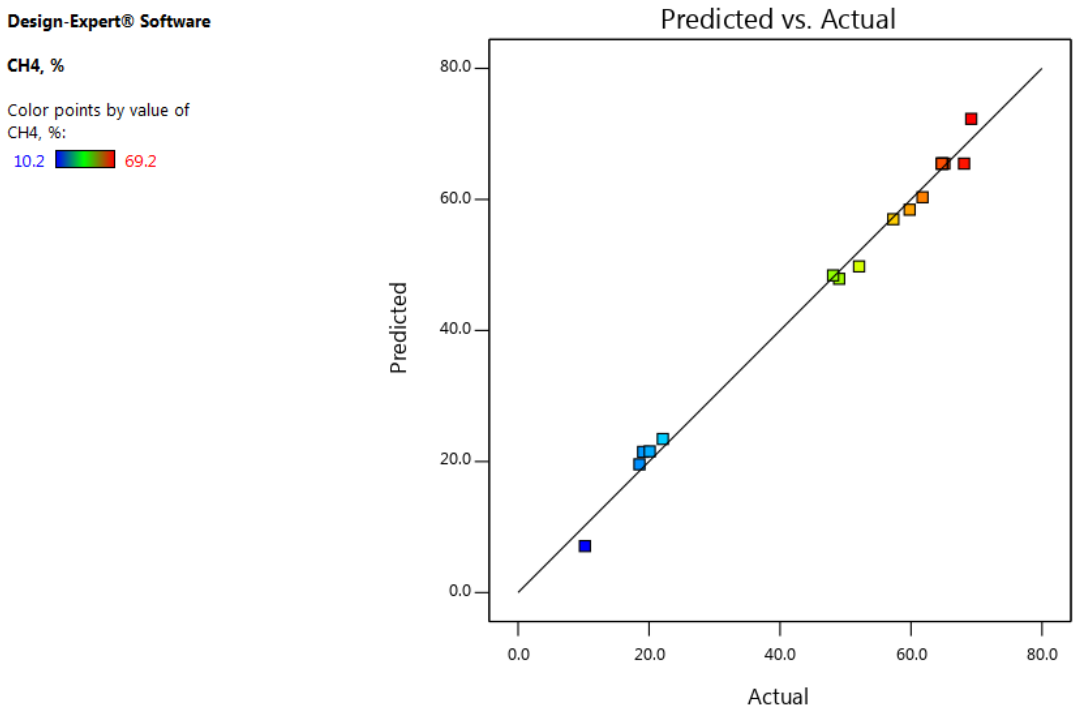


Figure 65: The scatter plot of the predicted values vs. the actual values of the methane concentration of the AD potato residues.

The perturbation plot in Figure 66 shows that, as the temperature deviates from approximately 35 ^{0}C to either a higher or lower value, the methane concentration decreases. Moreover, the methane concentration dramatically increases when the sludge concentration (C) is increased (direct relationship). While, the organic concentration (B) has an inverse relationship on the methane concentration. The methane concentration increases sharply when the organic concentration (B) is decreased (less sharp than when the sludge concentration is increased).

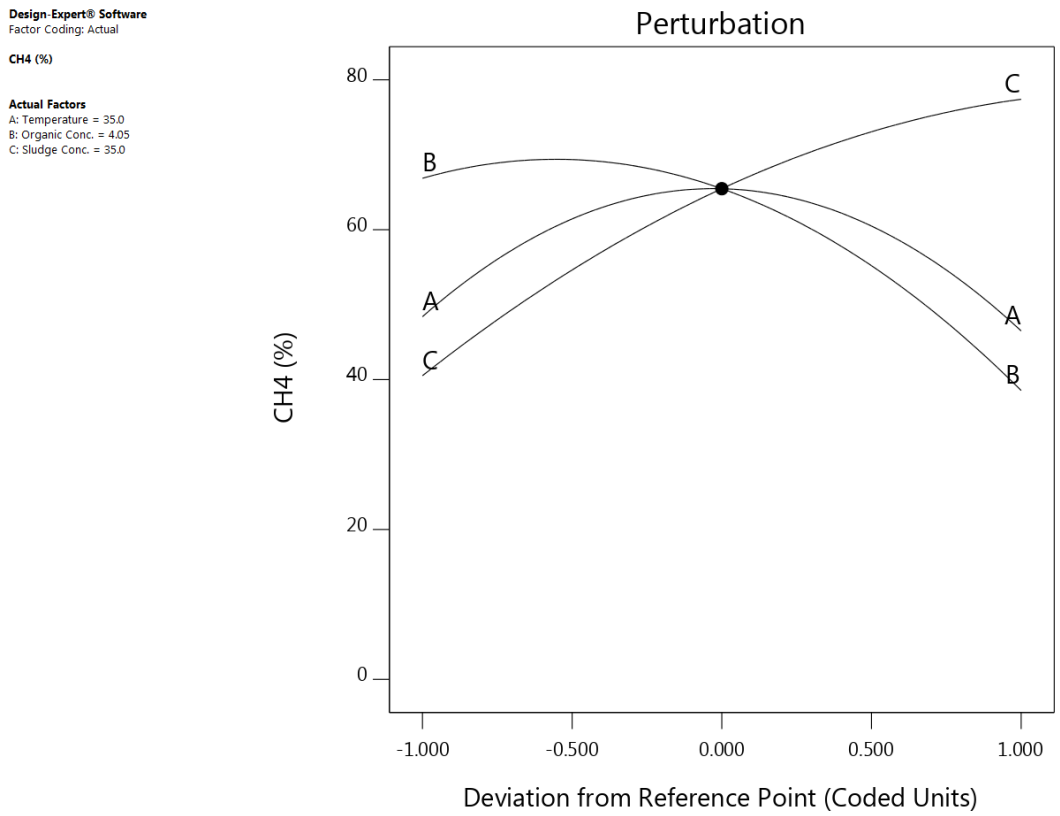


Figure 66: The Perturbation plot of the methane concentration response.

As aforementioned, the interaction of the organic concentration and the sludge concentration (BC) has significant impact on the methane concentration. Figure 67 and Figure 68 show the influence of the interaction on the methane concentration. As it is clear from the figures, the influence of the interaction was more significant when the sludge concentration was set to its lowest level and the organic concentration to its highest level. In other words, the rate of the increasing in the methane concentration decreased as the organic concentration decreased and sludge concentration increased.

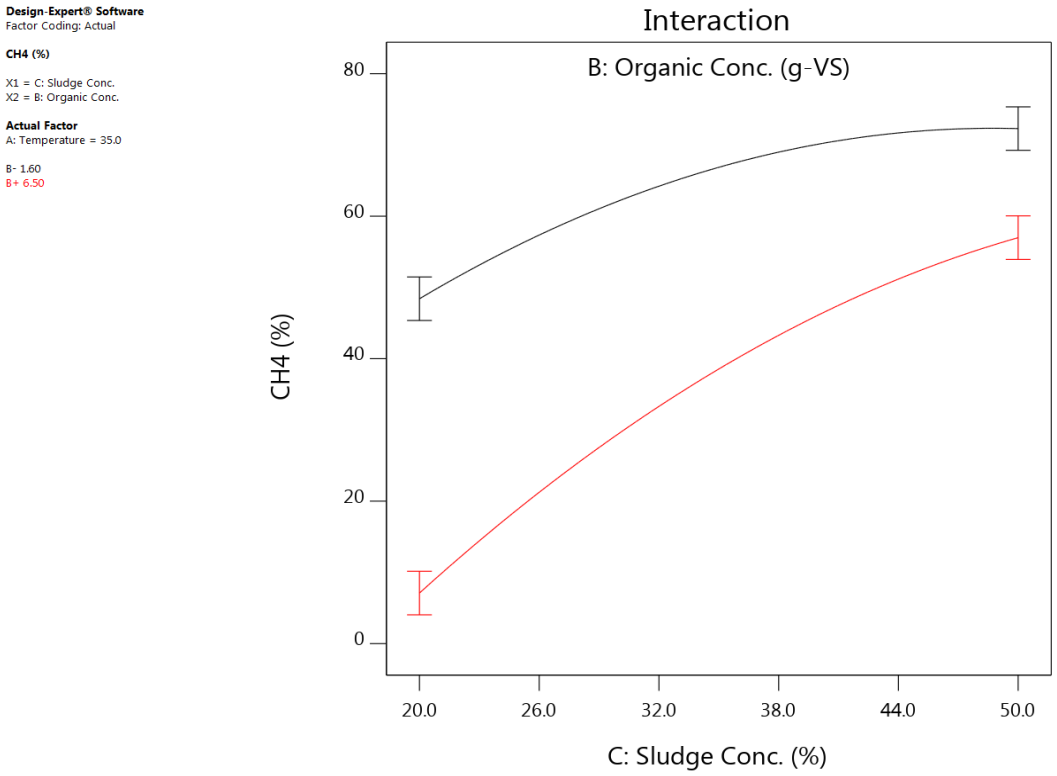


Figure 67: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the methane concentration.

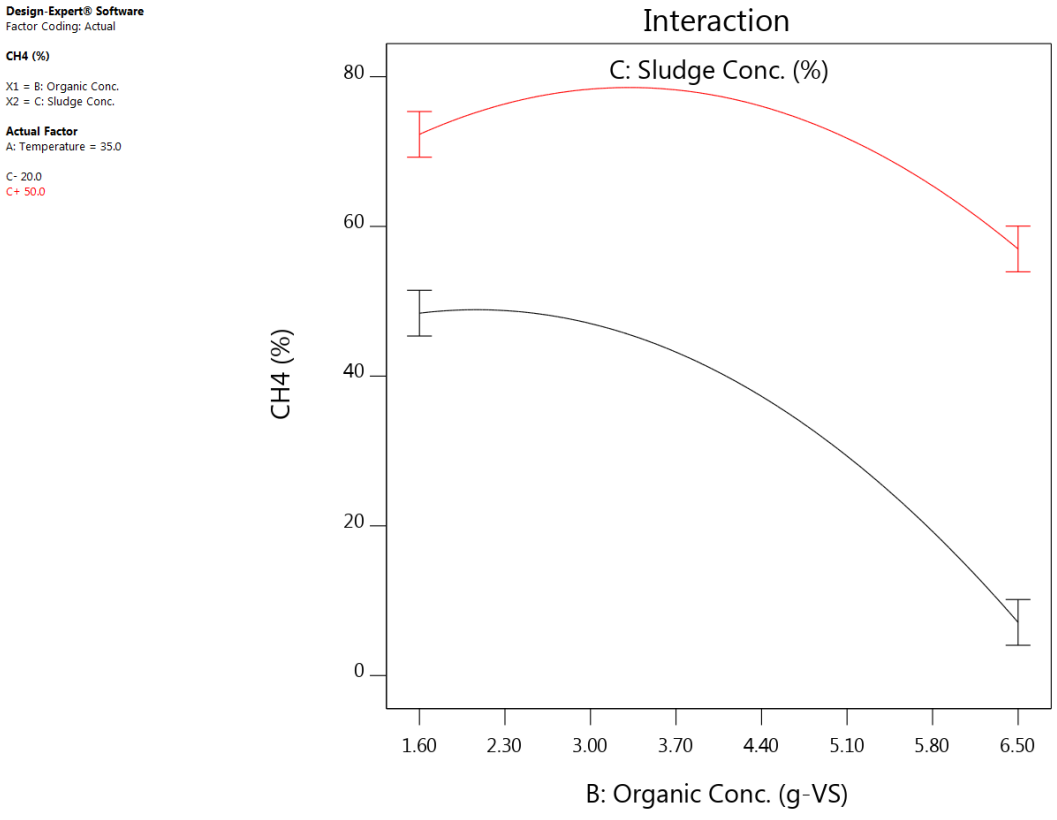


Figure 68: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the methane concentration in another view.

d. Carbon Dioxide Concentration

Looking at the concentrations of the methane concentration and carbon dioxide concentration which have resulted from each run, it can be noted that the there was an inverse relationship between the methane concentration and carbon dioxide concentration. The ANOVA (Table 21), confirmed that the developed model of the carbon dioxide concentration response was significant and the model terms which have significant influences on the carbon dioxide concentration were: B, C, BC, A^2 and B^2. The ANOVA also found that the "Lack of Fit" of the model was not significant (at $\alpha = 0.04$) and the values of the R^2 and adjusted R^2 were all close to 1 except the value of the predicted-R^2 which was a little less than 1. The "Adeq Precision" was greater than 4. Therefore, the regression of the model was good, hence the model adequately fit the data and can be used to navigate the design space. The analysis also revealed that the Pred. R^2 was in reasonable agreement with the Adj. R^2 (≤ 0.2). Equation 19 shows the mathematical model in an actual values of this response.

Table 21: The ANOVA table for the carbon dioxide concentration response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	3566.41	6	594.401	65.0114	0.0000002	Significant
A-Temperature, °C	36.41	1	36.409	3.9821	0.0739364	
B-Organic Conc., g	955.57	1	955.573	104.5138	0.0000013	
C-Sludge Conc., %	948.30	1	948.301	103.7184	0.0000013	
BC	195.07	1	195.068	21.3351	0.0009520	
A^2	487.09	1	487.095	53.2750	0.0000260	
B^2	867.33	1	867.326	94.8619	0.0000020	
Residual	91.43	10	9.143			
Lack of Fit	82.87	6	13.811	6.4515	0.0462886	not significant
Pure Error	8.56	4	2.141			
Cor Total	3657.84	16				
$R^2 = 0.98$				Adj $R^2 = 0.96$		
Pred $R^2 = 0.87$				Adeq Precision = 23.40		

$$\text{Carbon dioxide concentration, \%} = 1479.445 - 82.8284 * (\text{Temperature}) - 8.22908 * (\text{Organic concentration}) + 0.043759 * (\text{Sludge concentration}) - 0.19002 * (\text{Organic concentration}) * (\text{Sludge concentration}) + 1.193421 * (\text{Temperature})^2 + 2.387748 * (\text{Organic concentration})^2 \quad \text{Eqn. 19}$$

Furthermore, the perturbation plot in Figure 69 shows that, all factors were behaving in opposite manner to their behaviours found for methane concentration (as would be expected). For instance, when the temperature deviated from the reference point (35 ^{0}C), the carbon dioxide concentration increased. Figure 70, Figure 71 and Figure 72 illustrate the significant influence of the interaction of the organic concentration (g-VS) and sludge concentration (%) on the carbon dioxide concentration in an interaction, contour and 3D surface plots. In reference to Figure 68 and as shown in Figure 70, the organic concentration (B) and sludge concentration (C) affected methane concentration and carbon dioxide concentration in an opposite manner relatively. From Figure 70, it can be observed that the influence of the interaction was not significant when the organic concentration (B) was at its lowest level, however it was significant when it was set at its highest organic concentration. It can be also concluded that, at the lowest concentration of the sludge, the carbon dioxide concentration is increasing as the organic concentration is increasing.

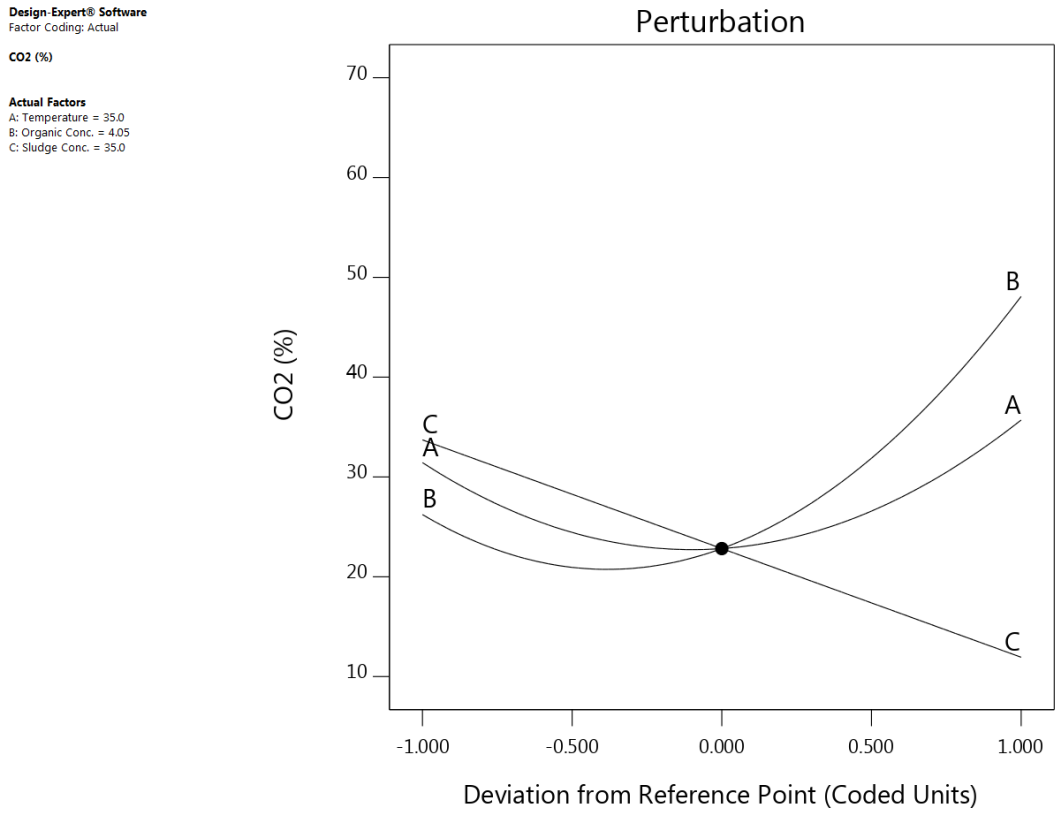


Figure 69: The Perturbation plot of the carbon dioxide concentration response.

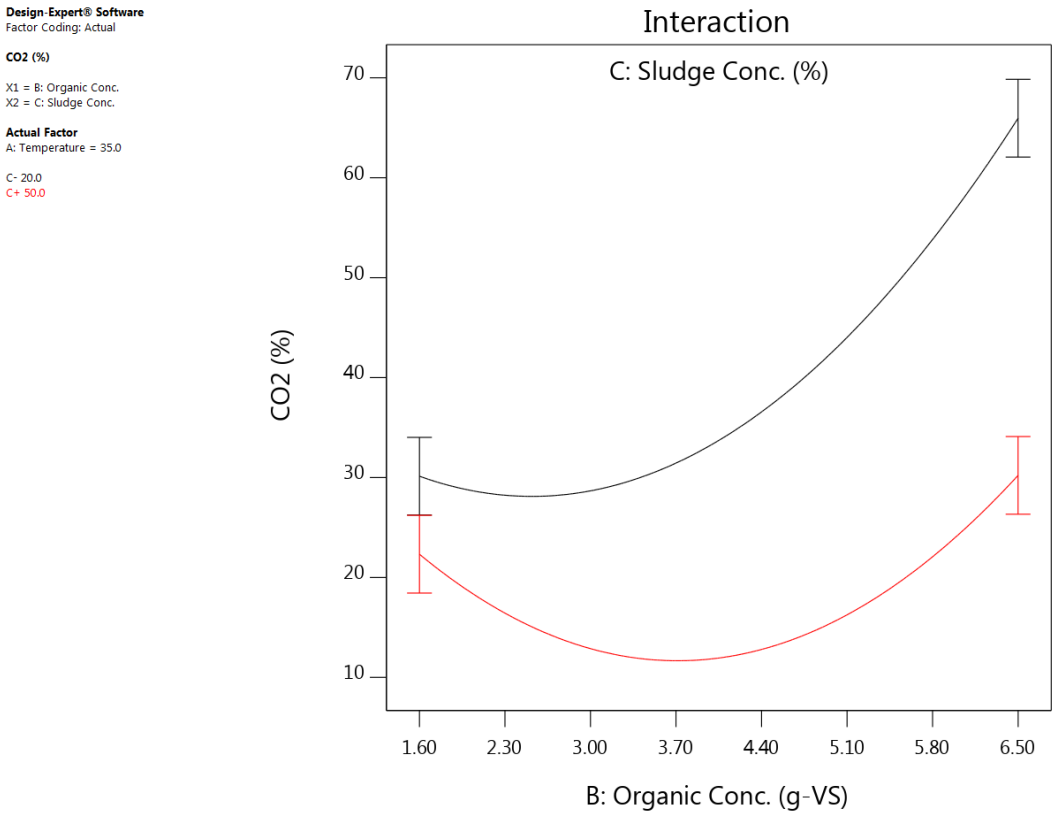


Figure 70: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the carbon dioxide concentration.

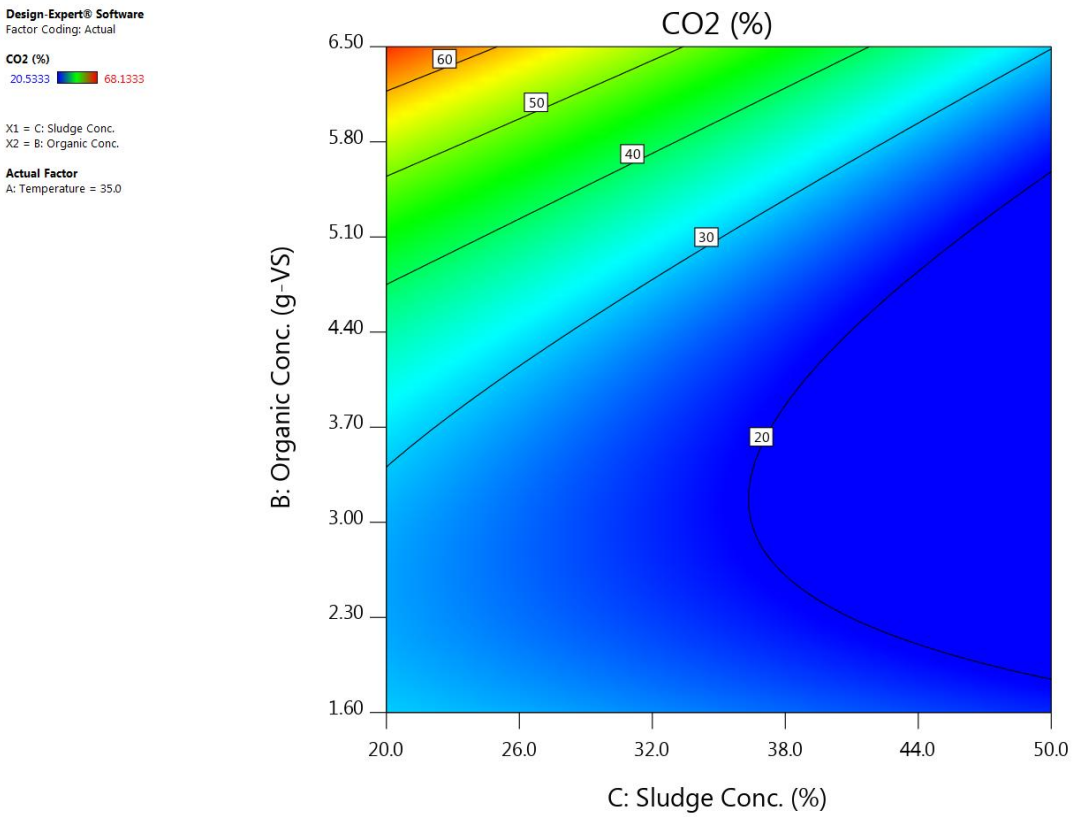


Figure 71: A contour plot shows the influence of the interaction of the organic concentration and sludge concentration on the carbon dioxide concentration.

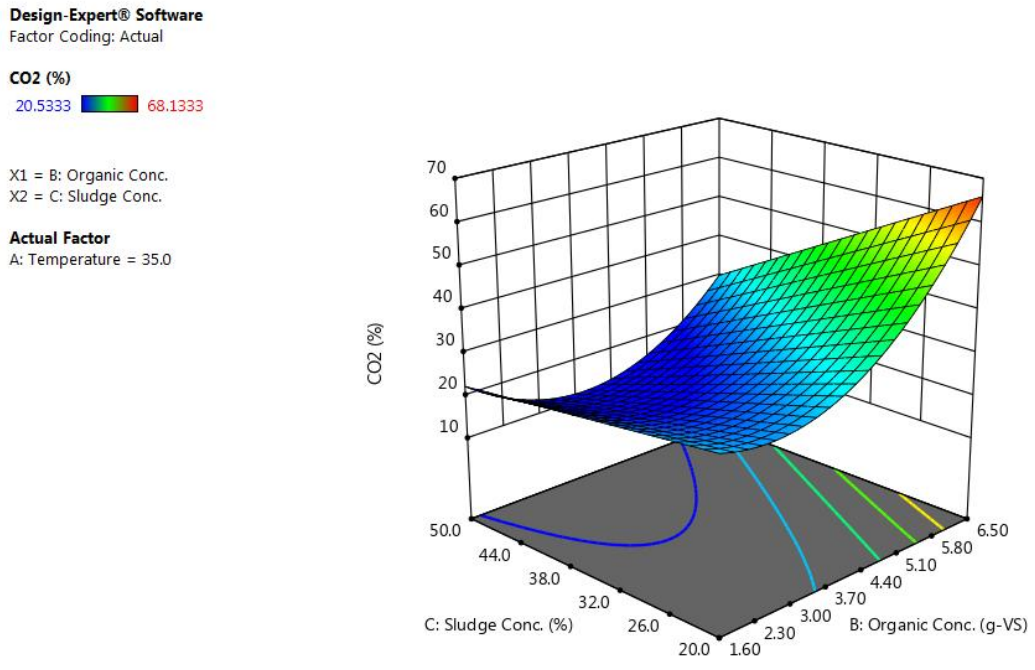


Figure 72: A 3D surface plot shows the influence of the interaction of the organic concentration and sludge concentration on the carbon dioxide concentration

e. Methane Content In One-Gram VS

This response was represented the content of the methane in the biogas produced from each gram VS in each run in cc/g-VS. It was calculated for each run by applying Equation 20;

$$CH_4 \text{ content} = \frac{\text{Total biogas volume}}{\text{Organic concentration}} * \frac{CH_4}{100} \qquad \text{Eqn. 20}$$

Furthermore, the ANOVA analysis of this response confirmed that the developed model was significant. The ANOVA revealed that, the model terms B, C, BC and A^2 have significant influence on the content of the methane which was produced from the one gram of the volatile solids (see Table 22). The lack of fit of the developed model was insignificant at $\alpha = 0.03$. Equation 21 and 22 show the final equations in terms of an actual and coded values. It is evident from Equation 22 that, the sludge concentration (C term) has the most significant influences on the methane content (positive influence). While, the organic concentration (B term) has the second most significant influence (negative influence).

Table 22: The ANOVA table for the methane content response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	756574.6	6	126095.8	28.71834	0.00001	Significant
A-Temperature, °C	1254.037	1	1254.037	0.285607	0.60473	
B-Organic Conc., g-VS	273354.4	1	273354.4	62.25654	0.00001	
C-Sludge Conc., %	312827.3	1	312827.3	71.24649	0.00001	
BC	43145.56	1	43145.56	9.826412	0.01061	
A^2	112758.8	1	112758.8	25.68084	0.00049	
C^2	17827.27	1	17827.27	4.060164	0.07158	
Residual	43907.75	10	4390.775			
Lack of Fit	40642.16	6	6773.693	8.297048	0.02999	not significant
Pure Error	3265.592	4	816.3979			
Cor Total	800482.3	16				
R^2= 0.95				Pred R^2= 0.67		
Adj R^2= 0.91				Adeq. Precision= 21.45		

$$\text{Methane content}, \frac{cc}{g} - VS = -22245.7 + 1275.216 * (\text{Temperature}, °C) + 23.46321 * (\text{Organic Conc.}, g - VS) + 4.412947 * (\text{Sludge Conc.}, \%) - 2.82606 * (\text{Organic Conc.}, g - VS) * (\text{Sludge Conc.}, \%) - 18.1578 * (\text{Temperature}, °C)^2 + 0.288795 * (\text{Sludge Conc.}, \%)^2 \quad \text{Eqn. 21}$$

$$\text{Methane content}, \frac{cc}{g} - VS = 346.2605 + 12.52017 * A - 184.849 * B + 197.7458 * C - 103.858 * B * C - 163.42 * A^2 + 64.97882 * C^2$$
Eqn. 22

In reference to Figure 66 and based on the perturbation plot in Figure 73, it is obvious that the behaviour of each factor on the methane content is similar up to an extent to their behaviour on the methane concentration response. From both figures, it is clear that the influence of the organic concentration (g-VS) on the methane content was sharper than its influence on the methane concentration. That is to say, the increase/decrease rate in the methane content was larger when the organic concentration increased or decreased. In contrast, the influence of the sludge concentration on the methane concentration was sharper than its influence on the methane content.

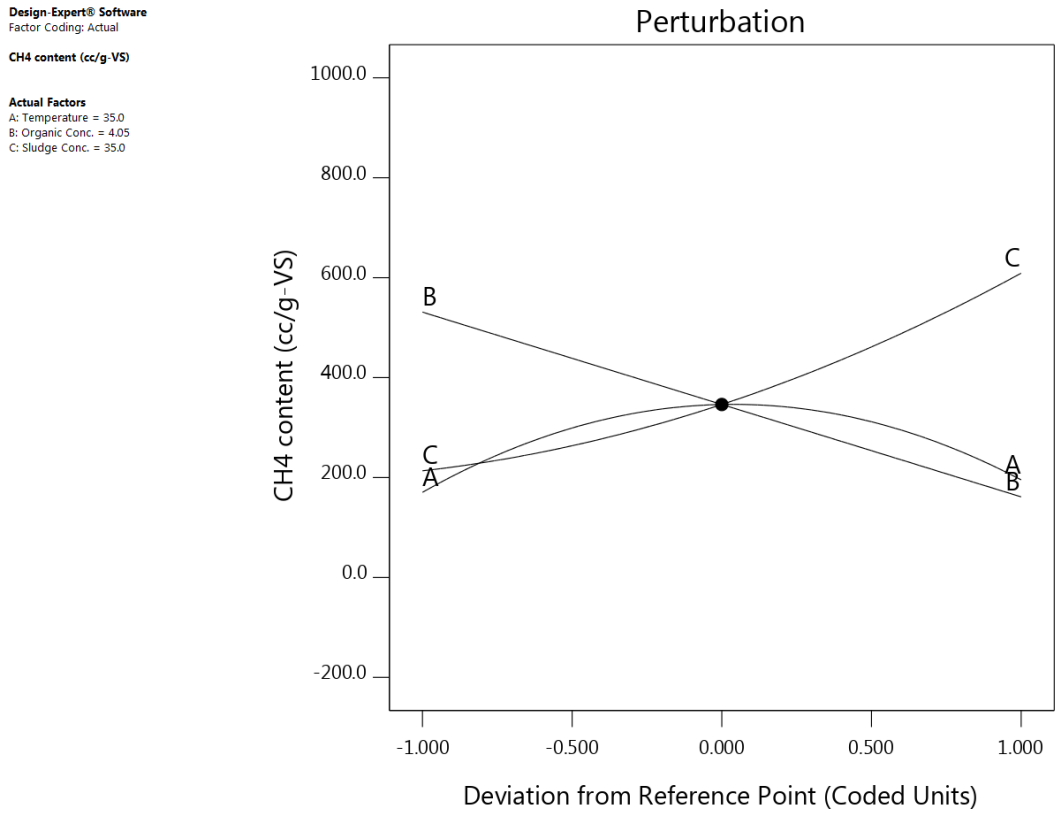


Figure 73: The Perturbation plot of the methane content response.

Figure 74 shows the influence of the interaction (BC) of the organic concentration and the sludge concentration on this response. According to Figure 74, the influence of the interaction was insignificant when the organic concentration, was set at its highest level, while it was significant when it was set to its lowest level.

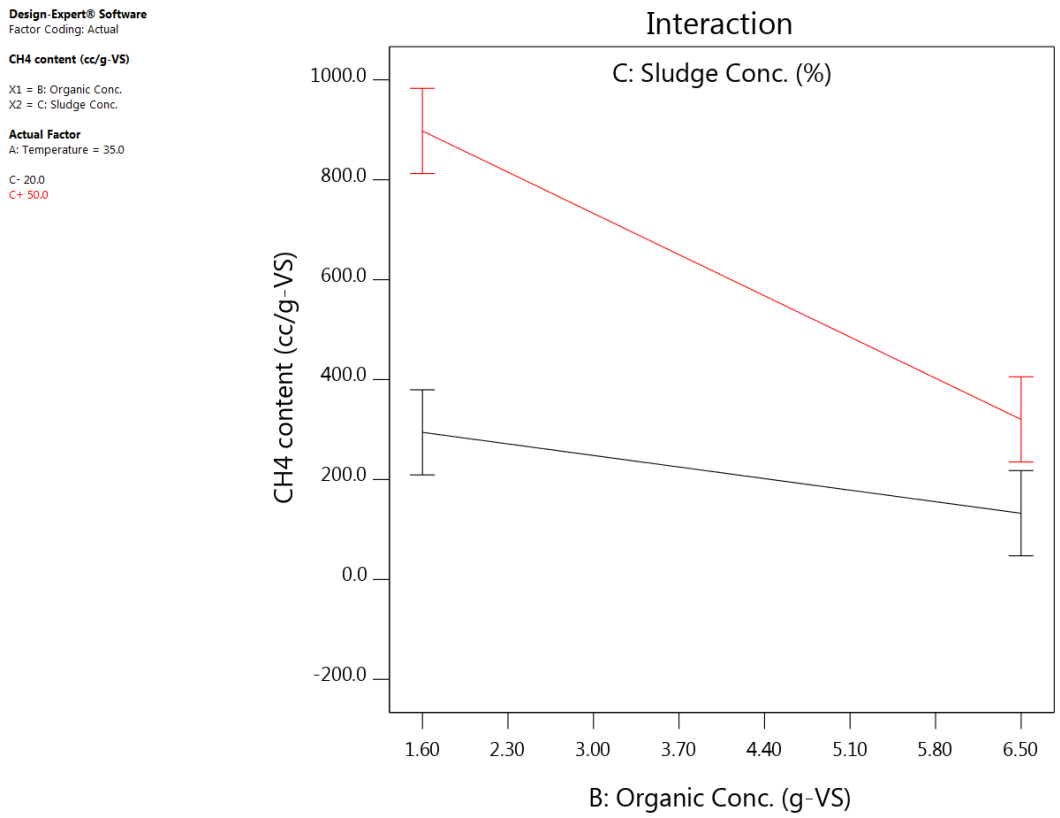


Figure 74: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the methane content.

2. Discussion Of The Key Findings

The biogas produced was collected twice and there were significant drops in the biogas volume from the first collection to the second one. This drop agrees with literature which confirms that the production of the biogas increases until reaches a peak and then decreases gradually [32, 33].

From the results obtained, the activity of the microorganisms was at the highest level at about the reference temperature (35 °C). The activity of the microorganisms reduces as the temperature deviating from the reference temperature. Regardless of the energy expenses, this finding explains the reason behind what was mentioned in Chapter 2, that most anaerobic digestion plants digest their substrates at temperatures between 35 to 37 °C [216]. On the other hand, the sludge concentration and the organic concentration (g-VS) respectively have the most significant influences almost on all responses.

From Figure 52 and Appendix H, it can be noted that, the pH levels decreased with the decreasing of the sludge concentration (C), lower than the organic

concentration (B) level. For instance, when the sludge concentration (C) was set to its lowest level and organic concentration (B) was set to its highest level, the pH level decreased and so on. It is well known, that the pH level has major impact not only on the hydrolysis rate but on the methanogenic bacteria as well [195]. Therefore, this decrease causes a reduction in the methane and increases the CO_2 production.

The interaction of the organic concentration (B) and sludge concentration (C) has shown a major impact on the quality of biogas. Additionally, the excessive feeding of the digester resulted in a negative impact on the methanogenic bacteria activity. Based on that, and according to Edwards, V. H. [228], careful determination of the concentrations of the sludge and substrates greatly helps in avoiding the inhibition of the growth of the bacteria and the distortion of the metabolism of it. This would also contribute, in avoiding the imbalancing of the bacterial population, VFA accumulation and digester failure [227, 229].

Furthermore, based on the differences between the results of the controls and the predicted results, it is obvious that the effect of the starch on the biogas quantity and quality was relatively low. Thus, the use of starch extracted from potato waste may help producing other bio-products which possibly increase the profitability of AD.

Compared to the findings of another study [115], when the performance of the AD of potato peels at different inoculum-to-substrate ratios were examined, the highest CH_4 content resulted from the potato waste in this study (967.4 CH_4/g-VS) was almost 4 times higher than what were found in that study. This can be attributed to imbalance effect of the inoculum and substrate in that study or might be due to using different inoculums.

4.2.2 AD Of Mango Residues

The mangoes were washed thoroughly prior to processing. The residues generated, constituted approximately one third of the total weight of the mango. Each fresh mango was found to contained approximately 90g peels, 25g seed and 30g seed coat. The starch represented approximately 20% of the seed weight. The peels and the leftovers of the separation of starch from the seeds, were used as the

substrates for the AD of mango residues. While, the seed coats were treated, so as to be used as a reinforcement material. Furthermore, the same procedure was applied on mango residues carried out to measure the TS, MS and VS% as used for the potato residues. In order to evaluate the impact of the fibres and starch on the quantity and quality of the biogas, the controls mango residues reactors contained all pre-treated residues of mango, including starch and fibres. Figure 75 and Figure 76 graphically show the TS and the adjusted VS% of the pre-treated biomass before and after the separation of starch and fibres. While, Figure 77, Figure 78, Figure 79 and Figure 80 depict the general results (see also Appendix H) and Table 23 shows the differences between the controls and the predicted results.

Figure 75: TS and VS before separation of the starch and fibres from mango residue.

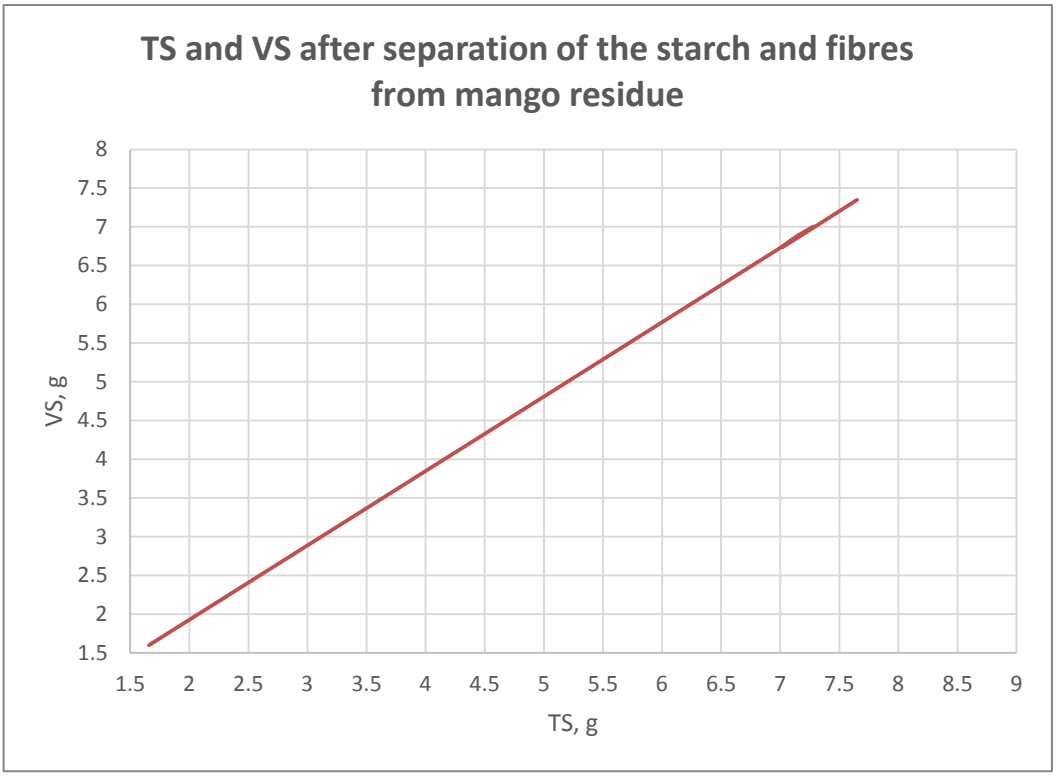


Figure 76: TS and VS after separation of the starch and fibres from mango residue.

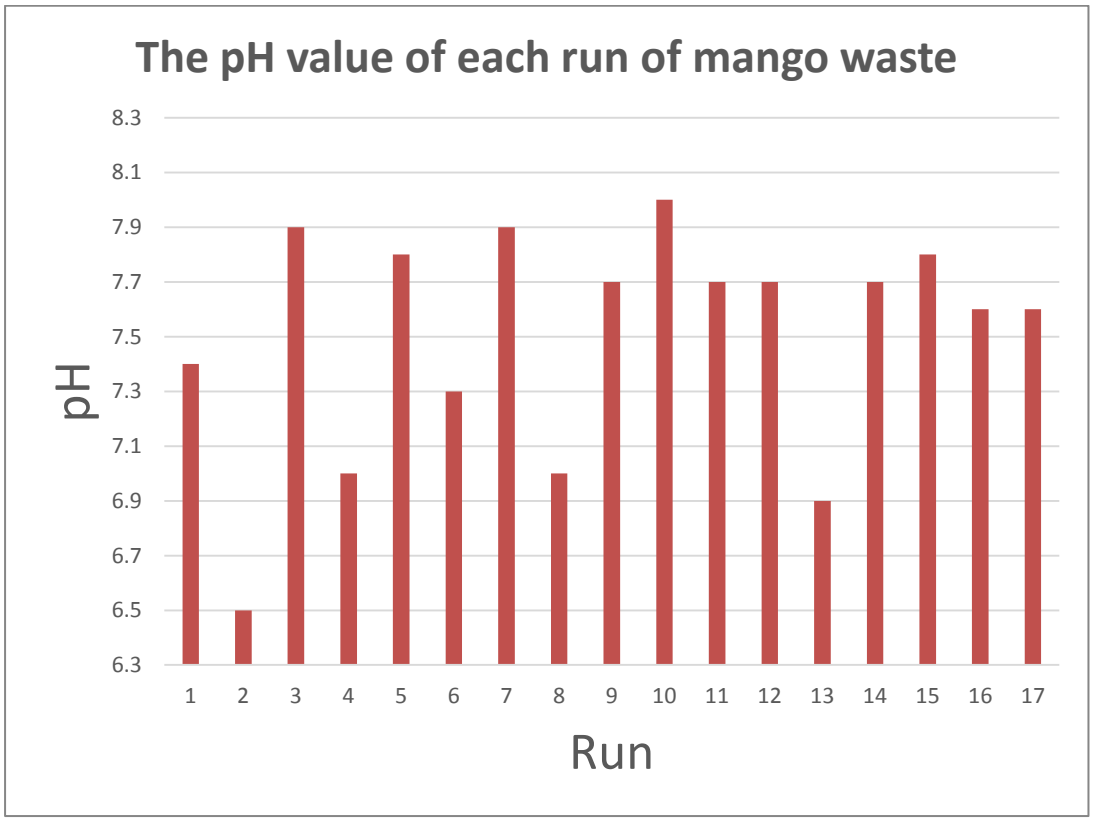


Figure 77: The pH value of each run of mango waste.

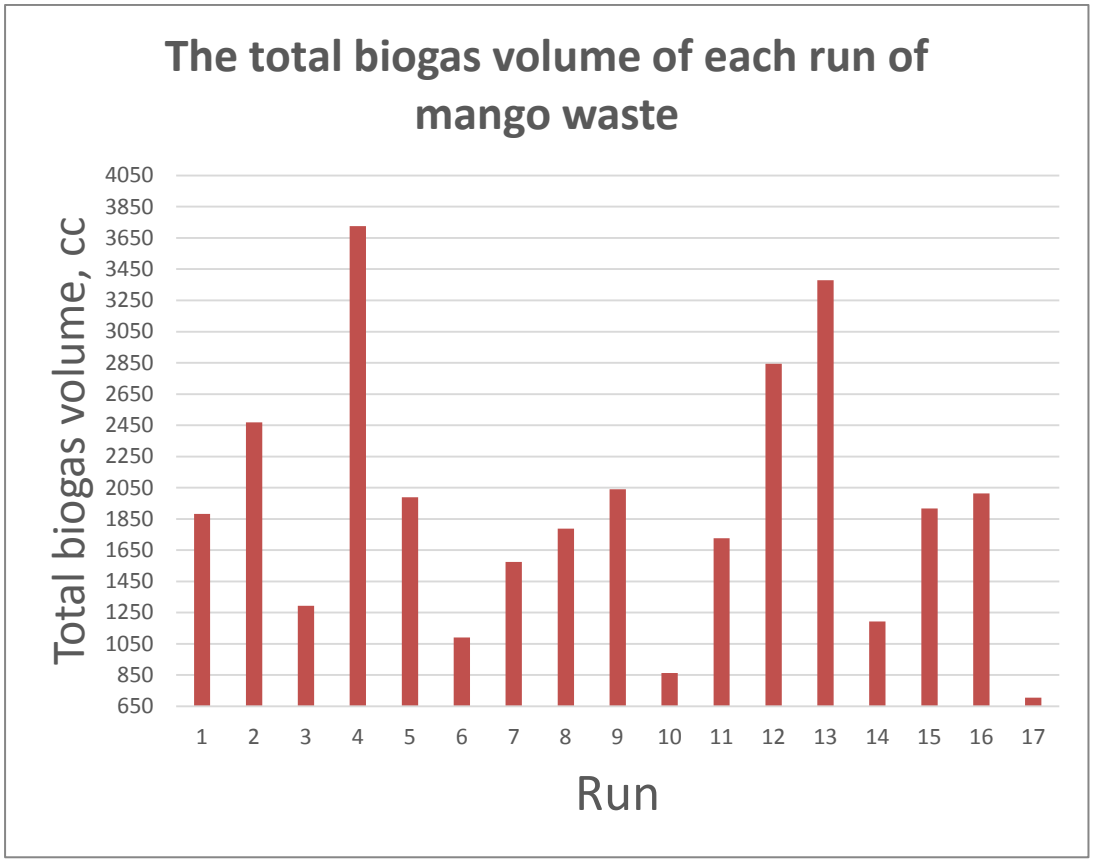


Figure 78: The total biogas volume of each runs of mango waste.

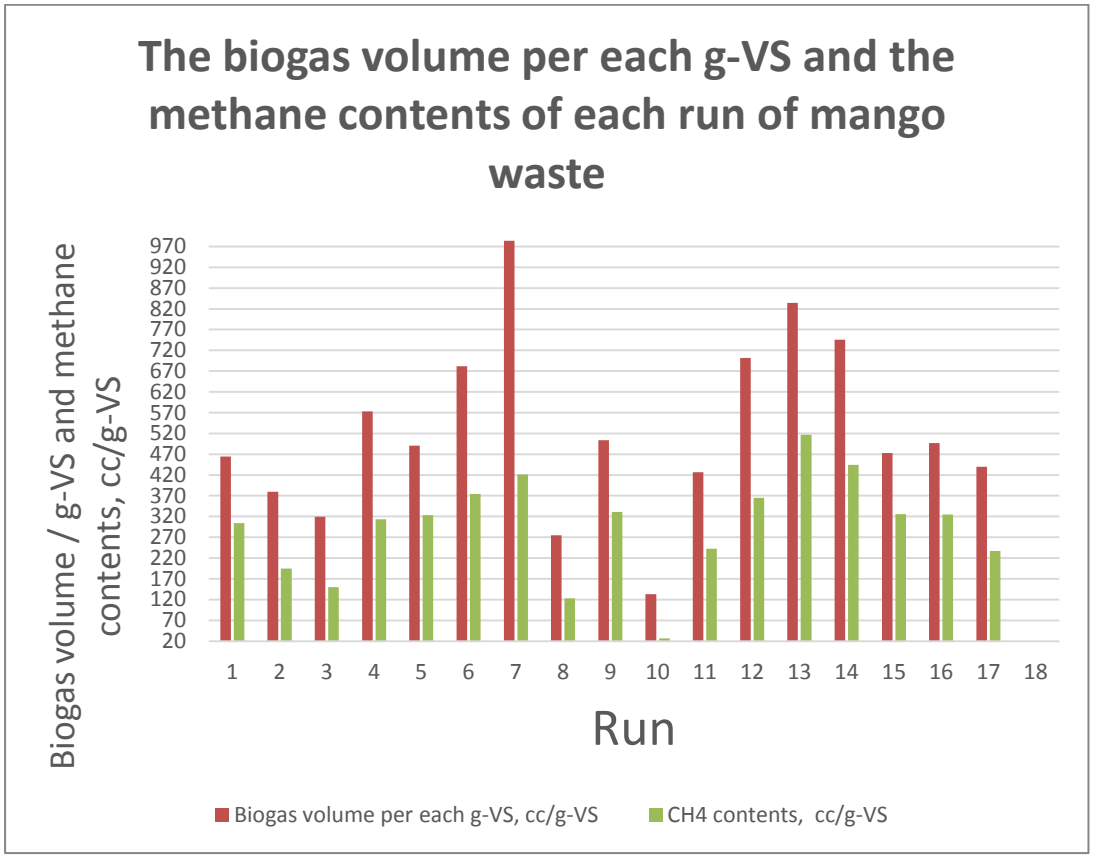


Figure 79: The biogas volume per each g-VS and the methane contents of each run of mango waste.

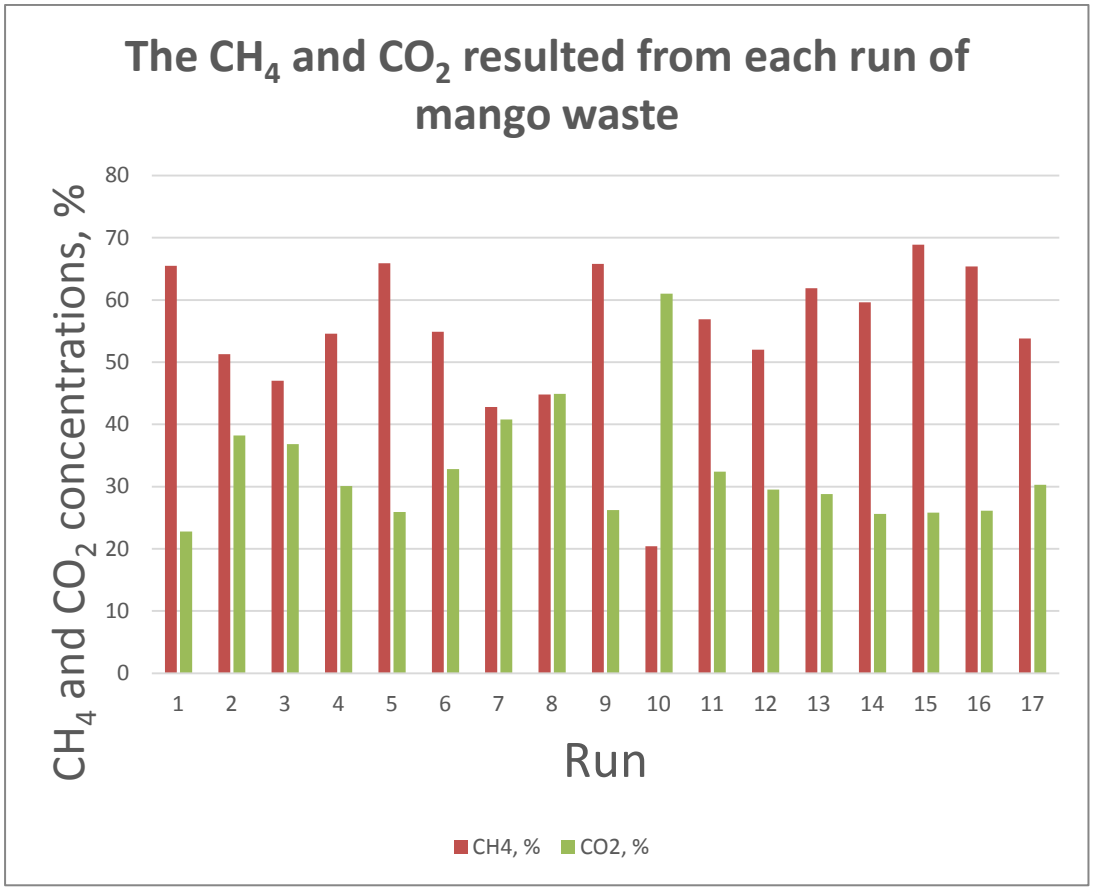


Figure 80: The CH_4 and CO_2 resulted from each run of mango waste.

Table 23: A comparison between the results of the controls and the predicted results at same conditions.

	Sample no.	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content/ g-VS
Actual (Controls)	1	32	6.5	50	7.9	2189.1	336.8	48.1	38.3	162.0
	2	35			7.8	2826.4	434.8	62.5	27.4	271.8
	3	38			7.8	3197.7	492	65.4	30.2	321.7
Predicted	1	32	6.5	50		3383.3	528.1	48.9	34.6	236.2
	2	35				3616.5	542.9	52.8	32.2	284.4
	3	38				4111.8	630.3	56.7	29.9	332.7
Difference %						-54.6	-56.8	-1.7	9.7	-45.8
						-28.0	-24.8	15.5	-17.6	-4.7
						-28.6	-28.1	13.3	1.2	-3.4

In order to check the equilibrium and stability of the systems and digesters, the pH level of every sample was measured to determine the average pH for each run. The pH of the three controls and of all samples ranged from a pH 6.5 to 8.

According to Figure 78, the highest total volume of the biogas produced was approximately 3725 cc at 35 ºC, 6.5 g-VS and 50% sludge. While, the lowest was approximately 704 cc at the condition of 35 ºC, 1.6 g-VS and 20% sludge. In terms of the volume of the biogas produced from the g-VS, the highest volume of mango residues recorded was approximately 984 cc/g-VS at the same condition of where the highest volume of potato was found, but at a lower volume. The lowest volume/g-VS recorded was 132.6 cc/g-VS at 35 ºC, 6.5 g-VS and 20% sludge (run 2) (see Figure 79 and Appendix H). On the other hand, Figure 80 reveals that the highest methane concentration was found at the centre points (run 5) and was 68.9%. Run 2 has also recorded the highest level of carbon dioxide concentration (61%).

Table 23 illustrates the impact of the application of the mango starch and seed coats on the quantity and quality of the AD biogas of mango residues. It is clear that, the impacts of the starch and coats was negative on the biogas quantity and relatively low on the biogas quality. This is can be due to the stiffness of the mango seed coats and therefore, they were difficult to digest by the microorganism.

1. Analysis Of The Responses

a. <u>Total Volume Of Biogas Produced</u>

The ANOVA in Table 24 shows that, the developed model as well as the following model terms A, B, C, AB, BC, A^2, B^2 and C^2 were significant. It is also clear that the model was adequately fit the data, as the lack of fit was not significant. The R^2, adjusted R^2, and predicted-R^2 were all close to 1 therefore, the regression of the model was good. The analysis also confirmed that the "Pred R-Squared" of 0.94 was in reasonable agreement with the "Adj R-Squared" of 0.99 and the “Adeq Precision” was greater than 4. Hence, the model adequately fit the data and can be used to navigate the design space. According to the table, sludge concentration (C term) has the highest influence on the total biogas volume. Equation 23 shows the mathematical model of this response.

Table 24: The ANOVA table for the total biogas volume response of the AD of mango residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	10876757.1	8	1359594.6	146.91	7.194E-08	significant
A-Temperature, °C	384564.5	1	384564.5	41.55	0.00019909	
B-Organic Conc., g-VS	2290870.13	1	2290870.13	247.54	2.6599E-07	
C-Sludge Conc., %	6011778.13	1	6011778.13	649.62	6.018E-09	
AB	84100.00	1	84100.00	9.09	0.01669708	
BC	993012.25	1	993012.25	107.30	6.5207E-06	
A^2	72339.60	1	72339.60	7.82	0.02334656	
B^2	905240.02	1	905240.02	97.82	9.2164E-06	
C^2	189818.55	1	189818.55	20.51	0.00192708	
Residual	74034.95	8	9254.37			
Lack of Fit	56469.75	4	14117.44	3.21	0.14216022	not significant
Pure Error	17565.20	4	4391.30			
Cor Total	10950792.00	16				
R^2= 0.99				Pred R^2= 0.94		
Adj R^2= 0.99				Adeq Precision= 40.06		

$$\text{Total biogas, cc} = 18950.34 - 1026.29 * (\text{Temperature, °C}) - 320.881 * (\text{Organic Conc., g} - \text{VS}) - 63.1742 * (\text{Sludge Conc., \%}) + 19.72789 * (\text{Temperature, °C}) * (\text{Organic Conc., g} - \text{VS}) + 13.55782 * (\text{Organic Conc., g} - \text{VS} * \text{Sludge Conc., \%} + 14.56389 * (\text{Temperature, °C})2 - 77.247 * (\text{Organic Conc., g} - \text{VS})2 + 0.943667 * (\text{Sludge Conc., \%})2 \quad \text{Eqn. 23}$$

Figure 81 illustrates the distribution of the residuals, where the distribution of the points was almost linear, therefore the residuals were normally distributed. As shown in the predicted values versus the actual values plot (Figure 82), there was a good correlation between the predicted and the actual results of the developed model.

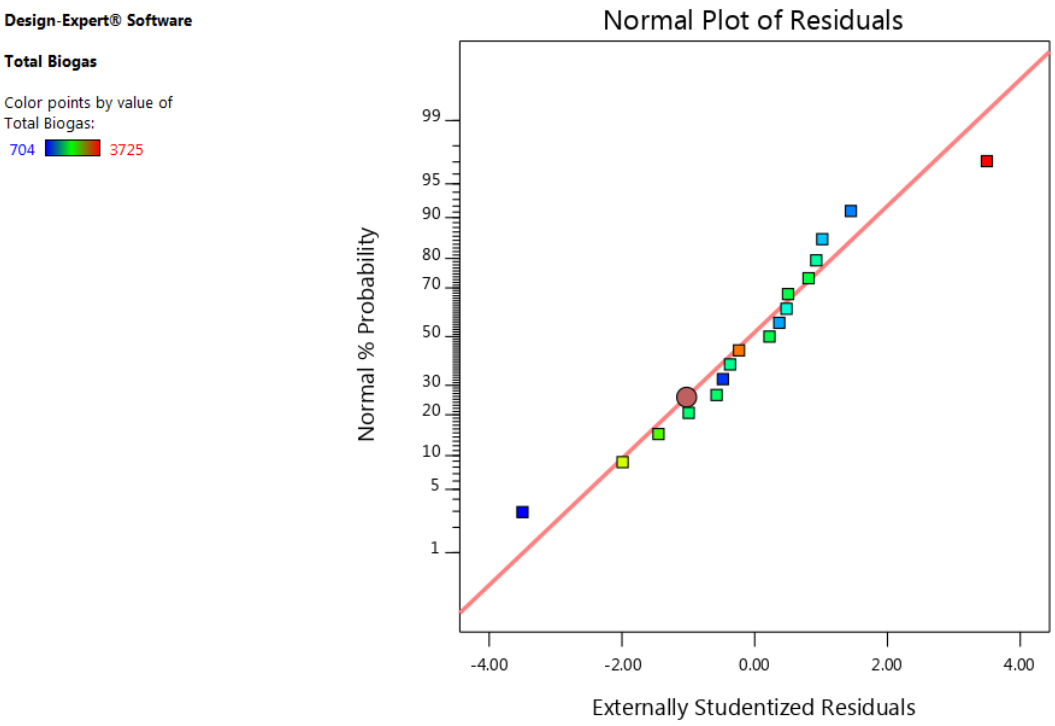


Figure 81: The normal plot of residuals.

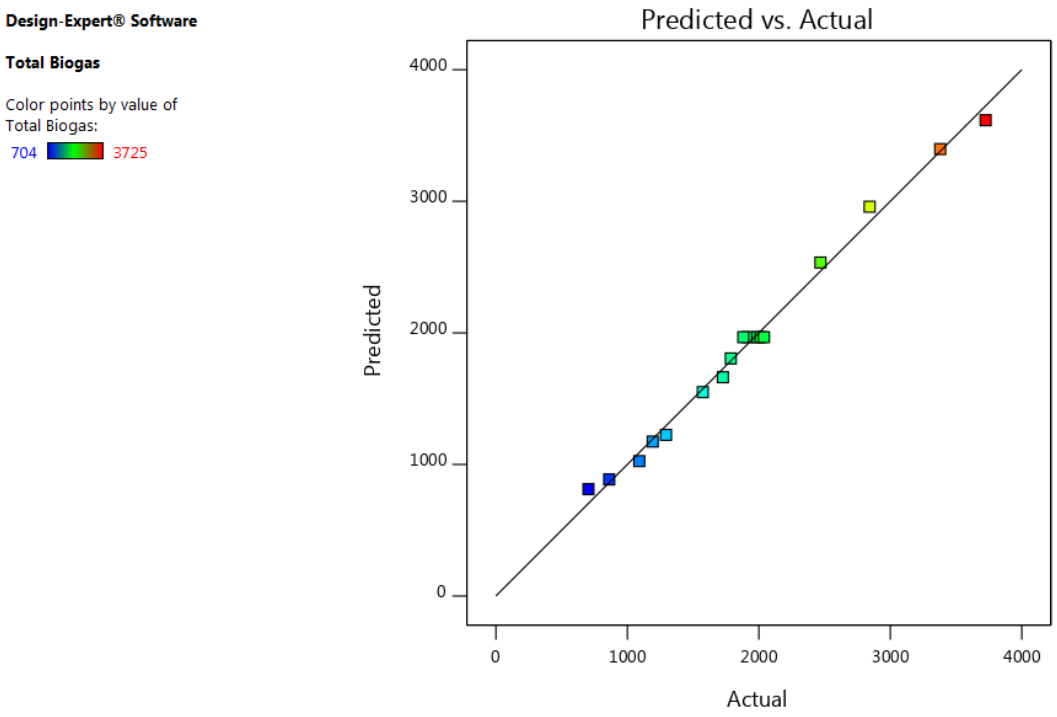


Figure 82: The scatter plot of the predicted values versus the actual values.

Figure 83 depicts the behaviour of each factor on the total biogas volume. In the figure, an increasing pattern can be observed in the total biogas volume when any of the parameters increased except organic concentration which the total biogas starts decreased when it reached approximately 5.3 g-VS. The extent of the influences of

the parameters on the biogas volume were different from one to another. Therefore, they varied with increase in volume. The plot also confirmed what has been previously stated that, the sludge concentration was the most significant influence on the total volume of the biogas.

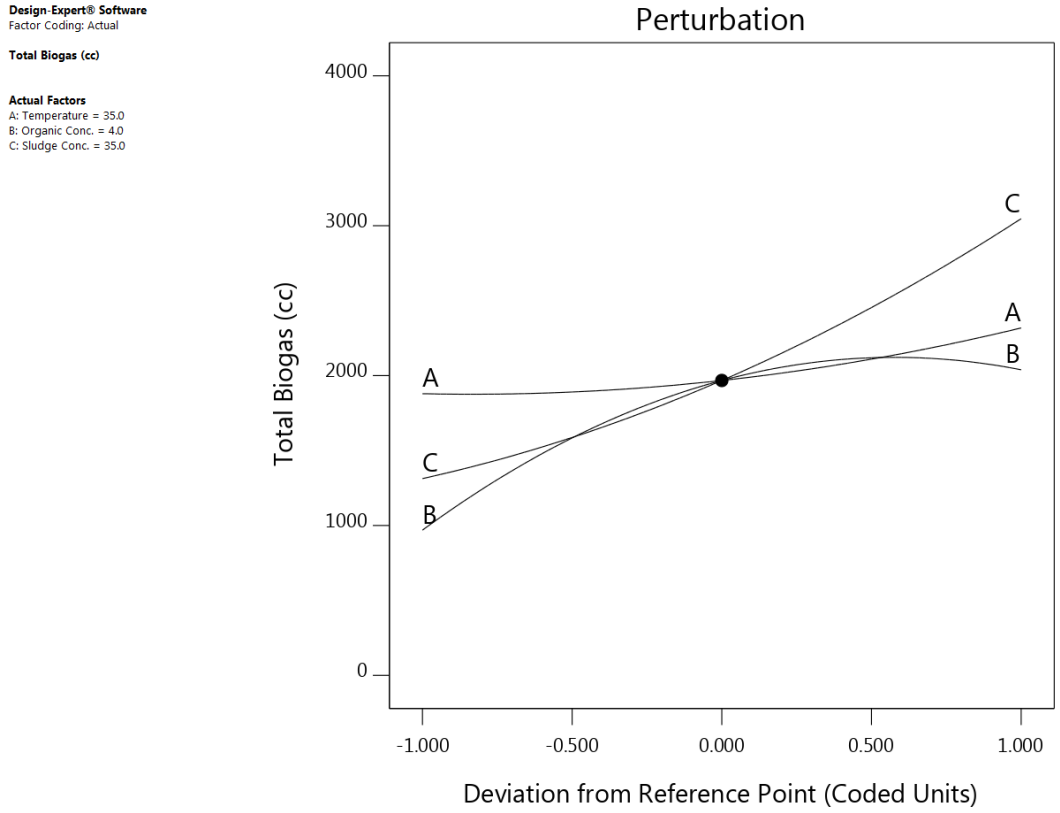


Figure 83: The Perturbation plot of the total biogas volume response.

The ANOVA (Table 24) revealed that, the interactions of the (AB) and (BC) were significant. Figure 84 depict the interaction of (AB) on the total biogas volume. It can be noted that, the influence of the interaction was insignificant when the organic concentration was set to its lowest level. The significance of the influence of the interaction increased as the organic concentration increased. It was at its highest influencing level when the organic concentration was set to its highest level. In addition, Figure 85 and Figure 86, show the interaction of the (BC) on the total biogas volume. According to these figures, the influence of the interaction of the organic and sludge concentrations was at its highest level when the organic concentration was at 6.5 g-VS. On the other hand, the influence of the interaction of (BC) was insignificant when the sludge concentration was set to its lowest level. The influence of the interaction dramatically increased as the sludge concentration increased.

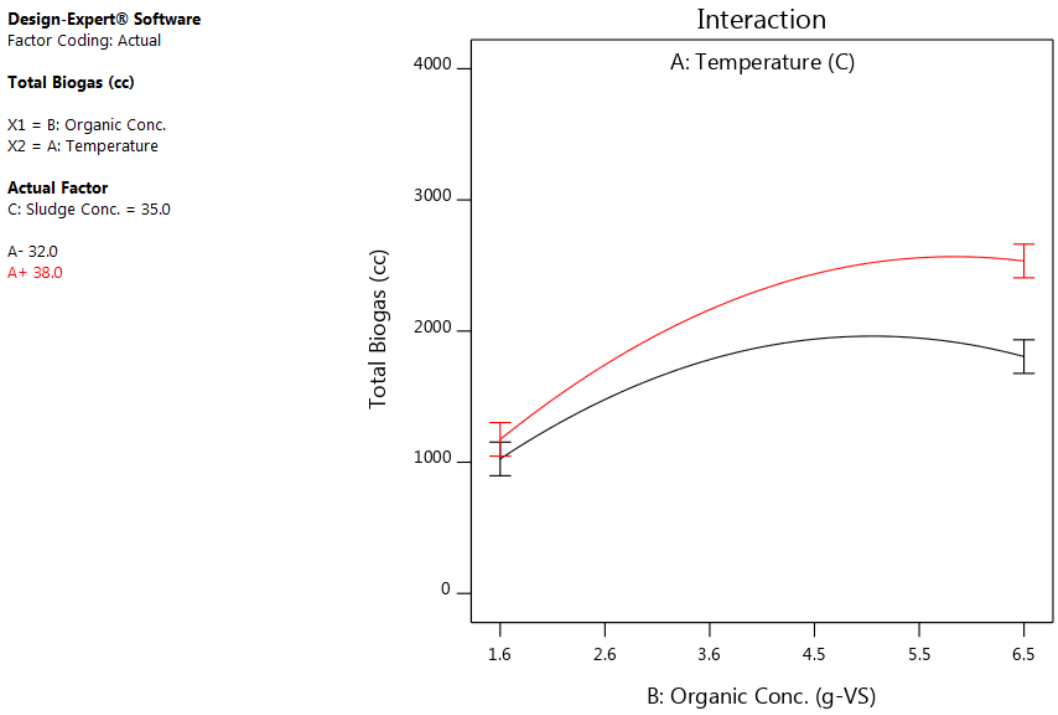


Figure 84: The interaction influence of the temperature and organic concentration.

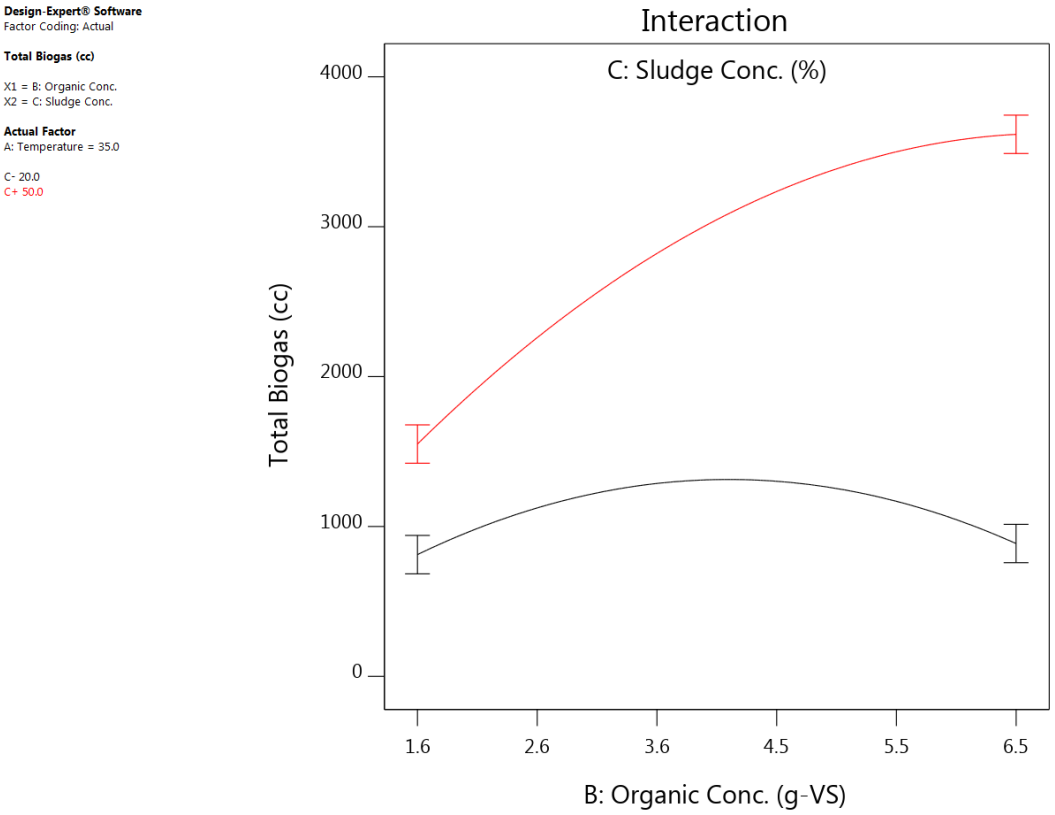


Figure 85: An interaction plot shows the influence of the interaction of the organic concentration and sludge concentration.

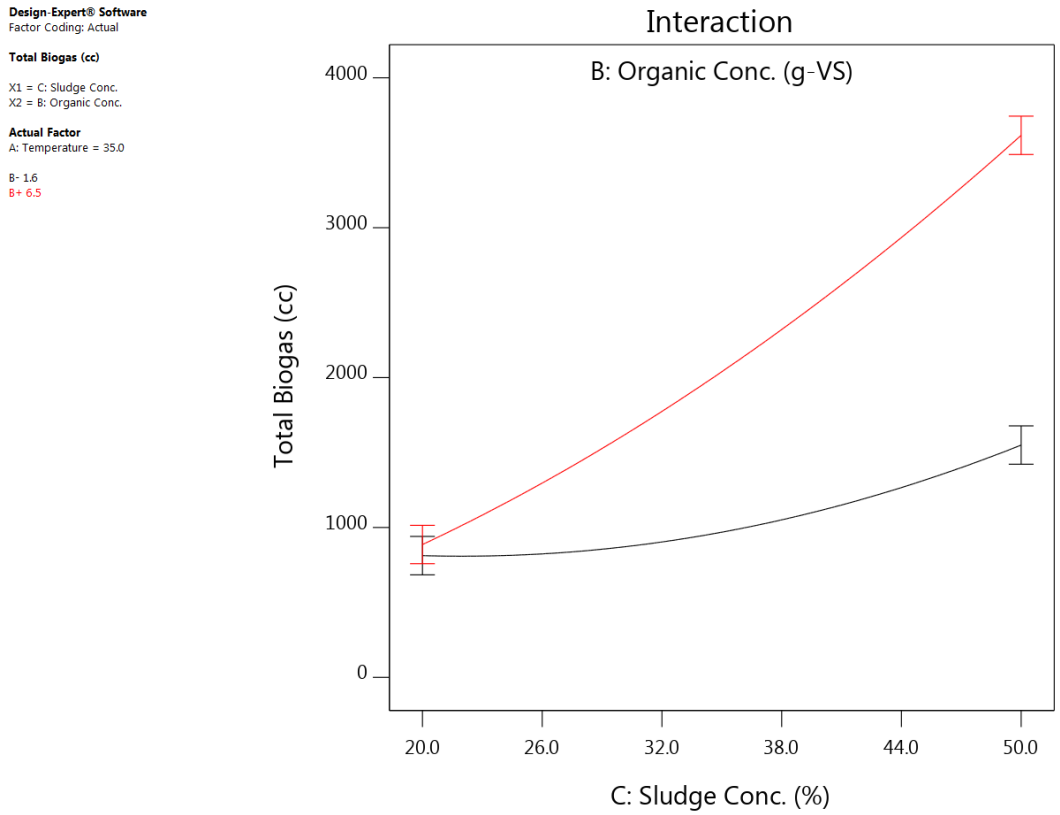


Figure 86: An interaction plot shows the influence of the interaction of the organic concentration and sludge concentration in another view.

b. The Volume Of Biogas Produced From Each Gram VS

The ANOVA (Table 25) checked the adequacy of the developed model and confirmed that, the model was significant, the lack of fit was insignificant and its regression was good. It was also shown that the model terms A, B, C, A^2 and C^2 had significant influences on the biogas volume produced from each g-VS produced. Equation 24 and 25 confirmed that the sludge concentration (C term) has the most significant influence (positive influence) following by the influence of the organic concentration (negative influence). While, the influence of the temperature term was less significant than the influences of the organic concentration and sludge concentration.

Table 25: The ANOVA for the biogas volume produced from each g-VS response of the AD of mango residues.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	711662.02	6	118610.34	161.60	2.29E-09	significant
A-Temperature, °C	20869.25	1	20869.25	28.43	0.000332	
B-Organic Conc., g-VS	278034.25	1	278034.25	378.81	2.8E-09	
C-Sludge Conc., %	393828.13	1	393828.13	536.57	5.08E-10	
BC	2678.06	1	2678.06	3.65	0.085183	
A^2	5575.69	1	5575.69	7.60	0.020262	
C^2	9804.95	1	9804.95	13.36	0.004426	
Residual	7339.77	10	733.98			
Lack of Fit	6265.58	6	1044.26	3.89	0.104812	not significant
Pure Error	1074.19	4	268.55			
Cor Total	719001.79	16				
R^2= 0.99				Pred R^2 = 0.95		
Adj R^2 = 0.98				Adeq Precision= 46.97		

$$\text{Biogas volume per 1g of VS, cc/gVS} = -280.411 + 17.02239 * (\text{Temperature}) - 76.0933 * (\text{Organic concentration}) + 14.79147 * (\text{Sludge concentration}) \quad \text{Eqn. 24}$$

$$\text{Biogas volume per 1g of VS, } \frac{\text{cc}}{\text{g}} - \text{VS} = 485.1158 + 51.075 * \text{A} - 186.425 * \text{B} + 221.875 * \text{C} - 25.875 * \text{BC} + 36.33947 * A^2 + 48.18947 * C^2 \quad \text{Eqn. 25}$$

Figure 87 and Figure 88 show the residual normality plot and the scatter plot of the predicted values versus the actual values. The figures proved that the residuals were normally distributed and there was a good correlation between the model's predicted results and the actual results.

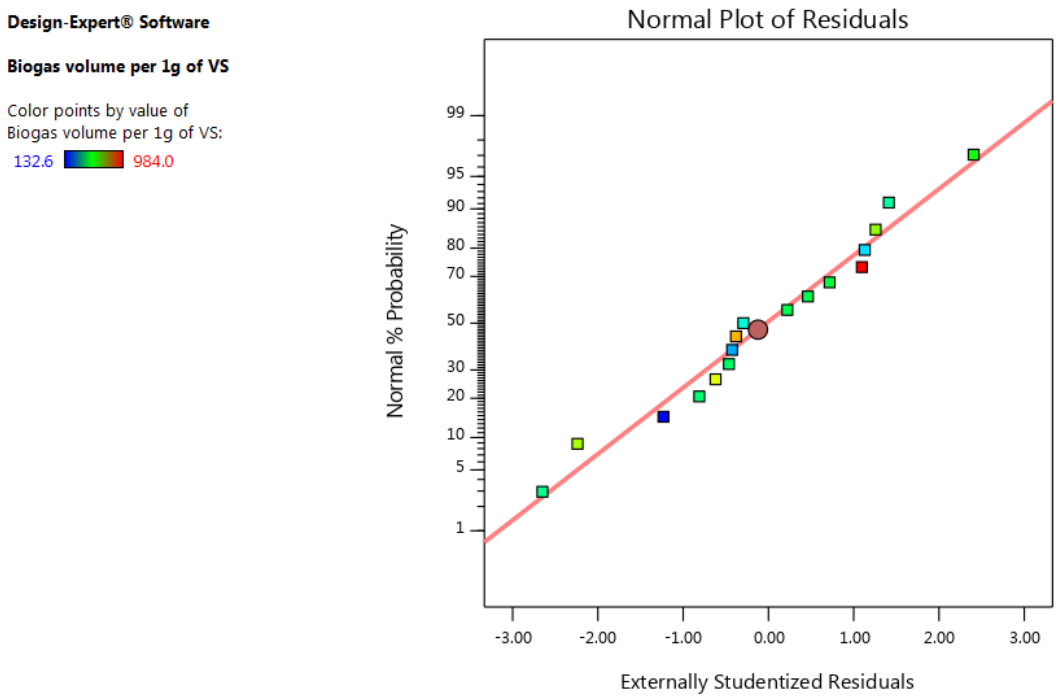


Figure 87: The normal plot of residuals.

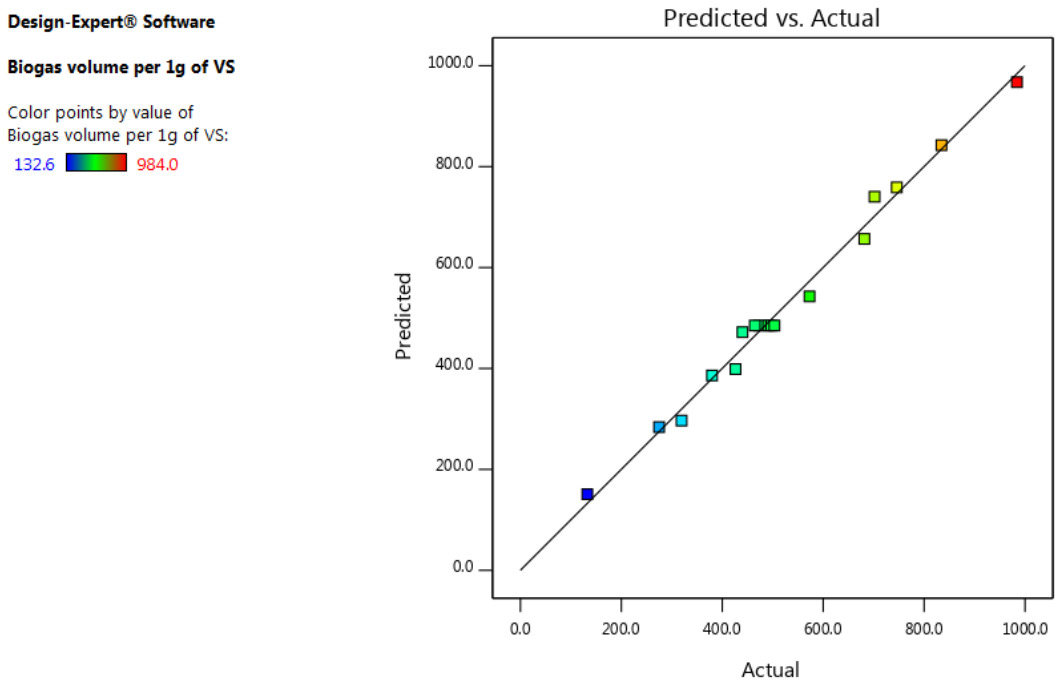


Figure 88: The scatter plot of the predicted values versus the actual values.

Figure 89 shows the influences of the factors and confirming what has been previously mentioned regarding to the impact of factors on the biogas volume produced from the one gram of the VS. As is evident, the temperature has slight positive impact on the volume and the increasing in the organic concentration (B term) was accompanied by a decrease in the biogas volume produced from each

gram VS. It is also clear that, the sludge concentration (C term) was in direct proportion to the biogas volume produced from the one gram VS.

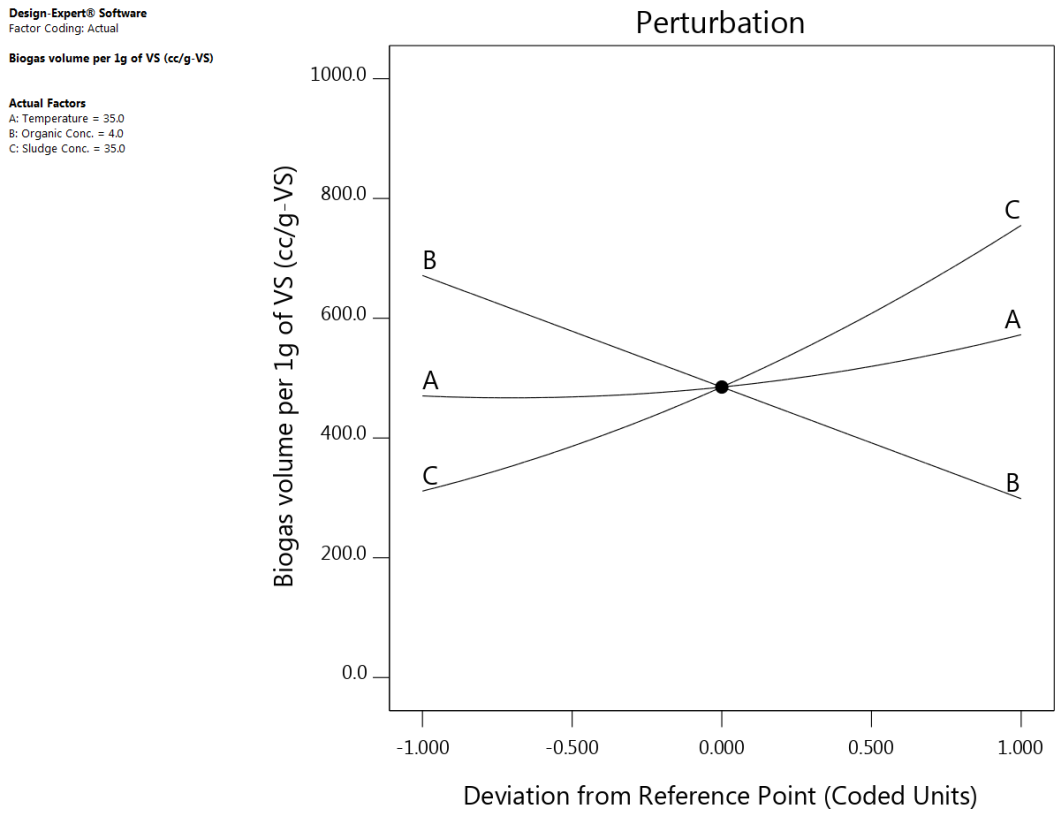


Figure 89: The Perturbation plot of the biogas volume produced from each g-VS response.

c. Methane Concentration

The ANOVA analysis in Table 26 revealed that, the developed model was significant and the lack of fit was insignificant. The analysis discovered that the following model terms had significant influences on the methane concentrations: A, B, C, BC, B^2 and C^2. The "Adeq Precision" was greater than 4 therefore, the model can be used to navigate the design space. The table showed that the interaction (BC) has the most significant influence. Equation 26 shows the final equation in terms of actual factors for this response as was computed by the software.

Table 26: The ANOVA table for the methane concentration response of the AD of mango residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2210.69	6	368.449	79.446	7.43E-08	significant
A-Temperature, °C	120.13	1	120.125	25.902	0.000471	
B-Organic Conc., g-VS	200.00	1	200.000	43.125	6.34E-05	
C-Sludge Conc., %	137.78	1	137.780	29.709	0.000281	
BC	510.76	1	510.760	110.132	1.02E-06	
B^2	676.21	1	676.213	145.807	2.75E-07	
C^2	497.53	1	497.533	107.280	1.15E-06	
Residual	46.38	10	4.638			
Lack of Fit	37.76	6	6.293	2.920	0.159492	not significant
Pure Error	8.62	4	2.155			
Cor Total	2257.07	16				
R^2= 0.98				Pred R^2 = 0.90		
Adj R^2 = 0.97				Adeq Precision= 31.81		

$$\text{Methane concentration}, \% = -30.8655 + 1.291667 * (\text{Temperature}) + 4.274769 * (\text{Organic concentration}) + 2.408554 * (\text{Sludge concentration}) + 0.307483 * (\text{Organic concentration}) * (\text{Sludge concentration}) - 2.10833 * (\text{Organic concentration})^2 - 0.04825 * (\text{Sludge concentration})^2 \quad \text{Eqn. 26}$$

Figure 90 is a perturbation plot of the methane concentration response. It shows the behaviour of each factor about the reference point. From the figure, it can be noted that the methane concentration increased as the temperature increased in the studied range. While, the methane concentration increased as the organic increased before it is reached approximately 4 g-VS and then began to decline gradually. In terms of the sludge concentration (C term) influence, the methane concentration increased as the sludge concentration increased until it is reached approximately 40% and then the methane concentration started to decrease slightly.

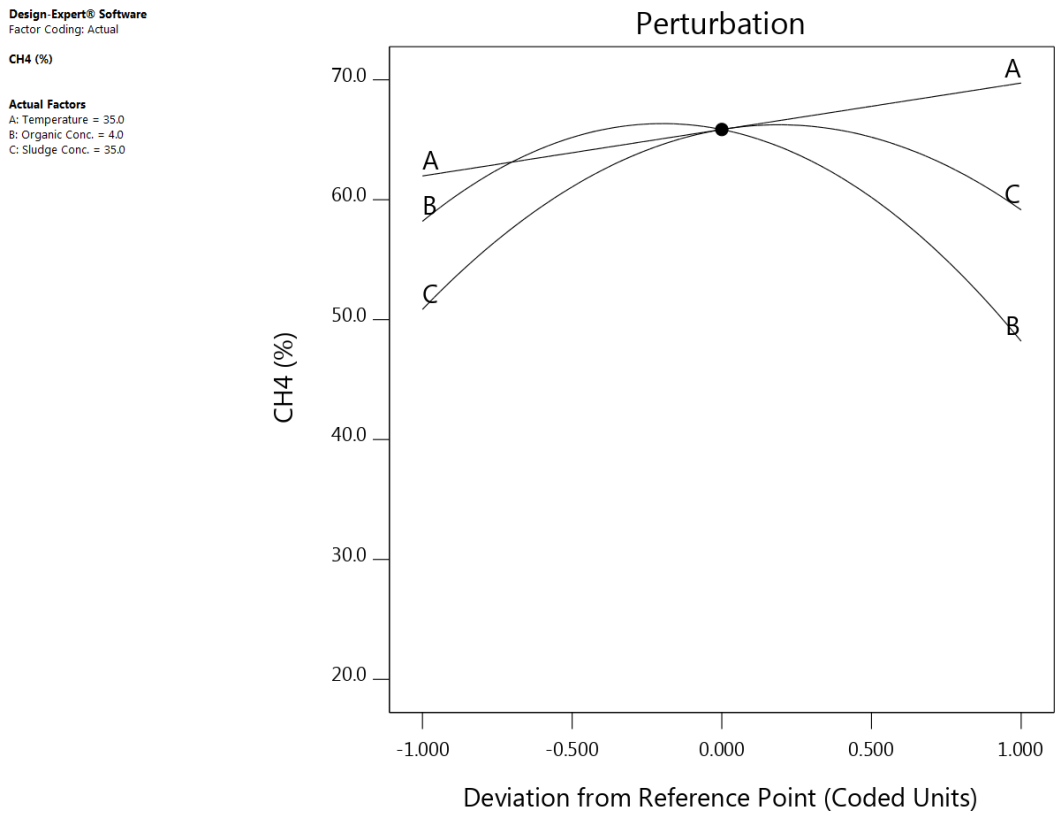


Figure 90: The Perturbation plot of the methane concentration response.

Looking at Figure 91 and Figure 92, at a temperature (A term) of 35 °C, it can be observed that, when the organic concentration (B term) was at the lowest level, changing in the sludge concentration (C term) did not make a big difference in the methane concentration. However, when the organic concentration was set at its highest level, a significant variation in the methane concentration was noted when the sludge concentration was increased/decreased. Additionally, when the organic concentration was set to 2.8 g-VS, the changing of the sludge concentration from its lowest to the highest level or vice versa did not make any changes in the methane concentration. Whereas, when the sludge concentration was approximately 40%, the changing in the organic concentration did not vary the methane concentration. In addition, Figure 93 illustrates the influence of this interaction in a 3D surface plot.

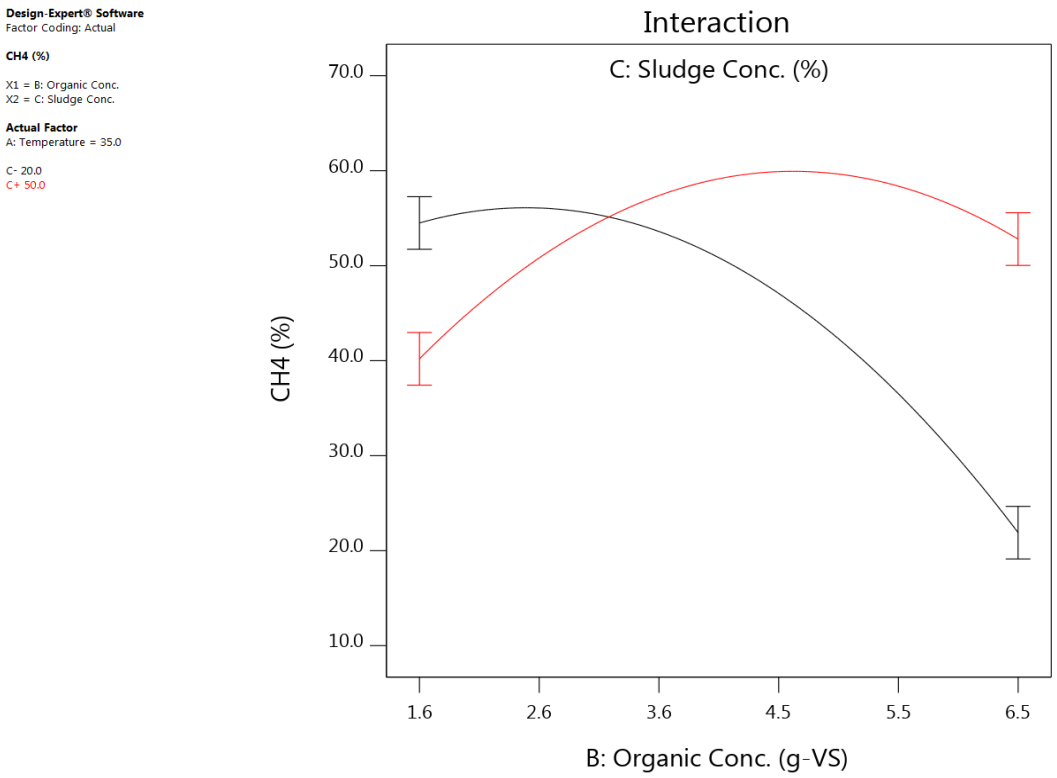


Figure 91: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the methane concentration.

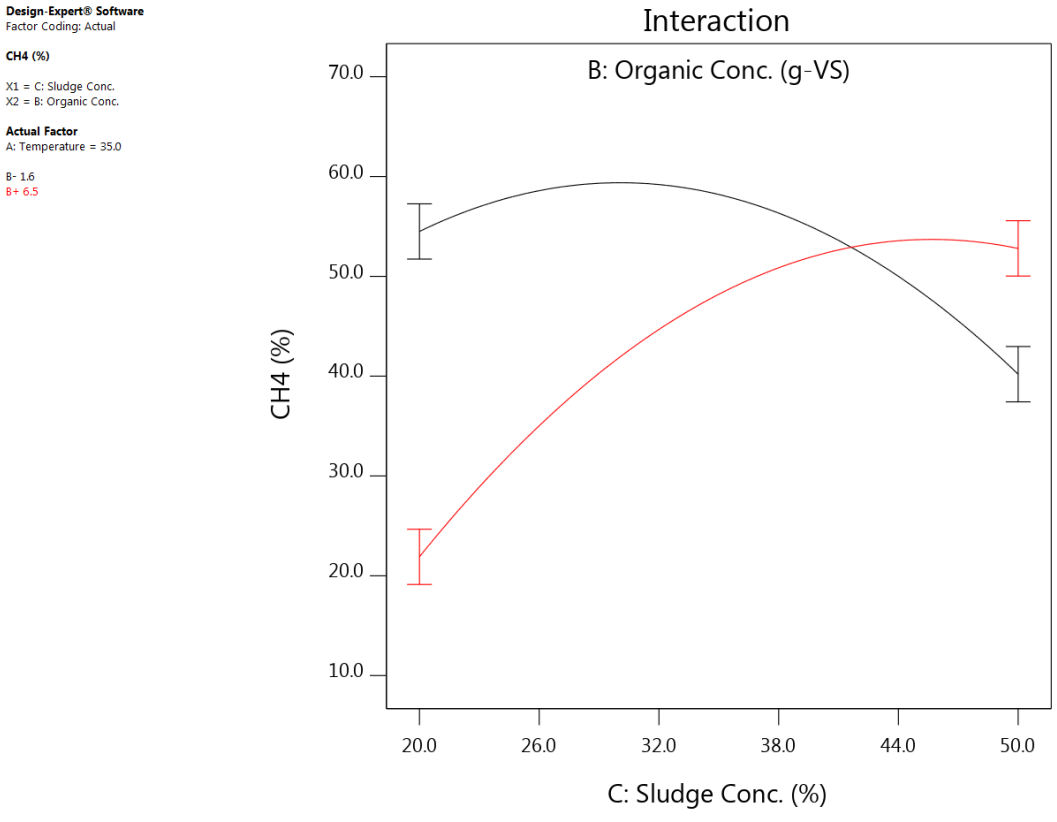


Figure 92: An interaction plot shows the interaction of the organic concentration and sludge concentration on the methane concentration in another view.

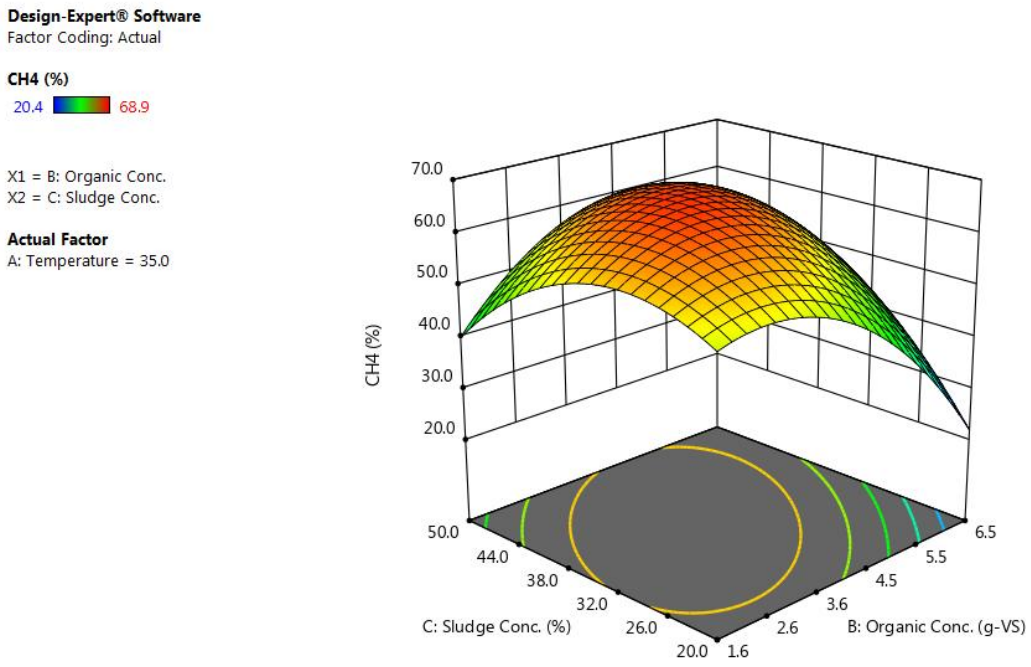


Figure 93: A 3D surface plot shows the interaction of the organic concentration and sludge concentration on the methane concentration.

d. Carbon Dioxide Concentration

The ANOVA (Table 27) checked the validity of the model and found it to be significant and the "lack of fit" insignificant. The same significant terms that had significant influences on the methane concentration also significantly influencing the carbon dioxide concentration but to an opposite effect. It was also confirmed that the model can be used to navigate the design space. The regression of the model was good and the Pred. R^2 was in reasonable agreement with the Adj. R^2 and the difference between them was less than 0.2. In reference to Table 26 and according to Table 27, model term (BC) have the most significant influences on the methane concentration and carbon dioxide concentration. Obviously, the same terms have significant effect on both responses but in different amounts. In comparison between the results of the methane concentration and carbon dioxide concentration were measured from each sample, it is evident that the increase in methane concentration was associated with a decrease in carbon dioxide concentration and vice versa. Equation 27 illustrates the mathematical model of this response.

Table 27: The ANOVA table for carbon dioxide concentration response of the AD of mango residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1390.90	6	231.817	62.00	2.47E-07	significant
A-Temperature, °C	45.13	1	45.125	12.07	0.005981	
B-Organic Conc., g-VS	249.76	1	249.761	66.80	9.76E-06	
C-Sludge Conc., %	122.46	1	122.461	32.75	0.000192	
BC	428.49	1	428.490	114.60	8.48E-07	
B^2	371.51	1	371.511	99.36	1.64E-06	
C^2	145.99	1	145.994	39.05	9.52E-05	
Residual	37.39	10	3.739			
Lack of Fit	29.10	6	4.850	2.34	0.215096	not significant
Pure Error	8.29	4	2.073			
Cor Total	1428.29	16				
R^2= 0.97				Pred R^2 = 0.89		
Adj R^2 = 0.96				Adeq Precision= 28.63		

$$\text{Carbon dioxide concentration, \%} = 70.96908 - 0.79167 * (\text{Temperature}) - 0.52033 * (\text{Organic concentration}) - 0.94964 * (\text{Sludge concentration}) - 0.28163 * (\text{Organic concentration}) * (\text{Sludge concentration}) + 1.562726 * (\text{Organic concentration})^2 + 0.026135 * (\text{Sludge concentration})^2 \quad \text{Eqn. 27}$$

The perturbation plot of the carbon dioxide concentration response in Figure 94 clearly shows that the influencing factors have an opposite effect to the behaviour they have on the methane concentration (see Figure 90).

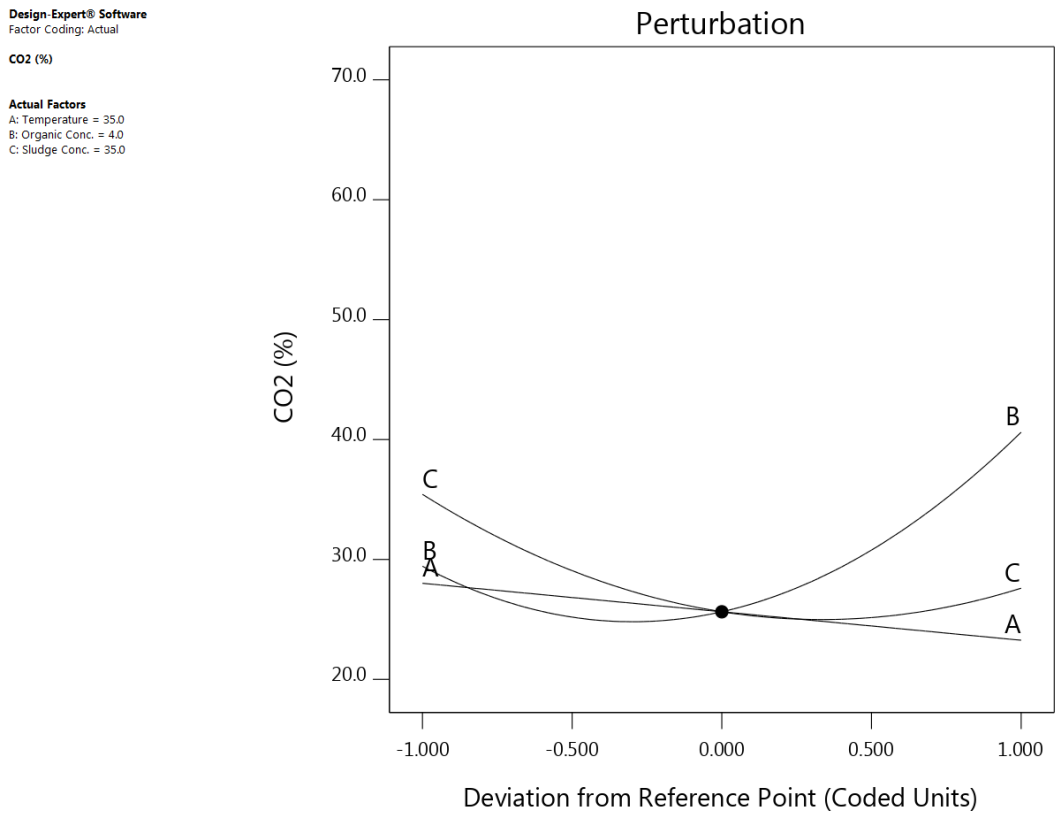


Figure 94: The Perturbation plot of the carbon dioxide concentration response.

Relatively, the same concept of the effect of the interaction BC had on the methane concentration, is applicable to its effect on the carbon dioxide concentration but in an opposite way. For instance, when the organic concentration was set to its highest level, a significant variation in the carbon dioxide concentration can be observed when the sludge concentration increased or decreased. Figure 95 and Figure 96 illustrate the influence of BC interaction at mid temperature level of 35 °C.

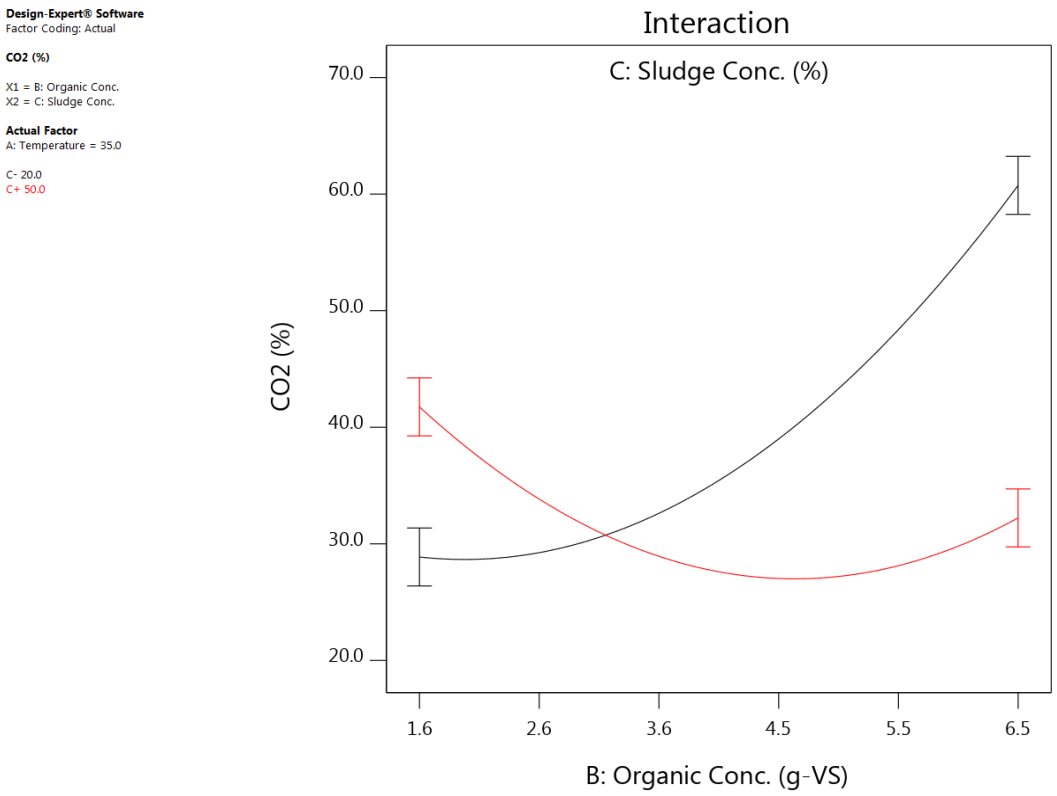


Figure 95: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the carbon dioxide concentration.

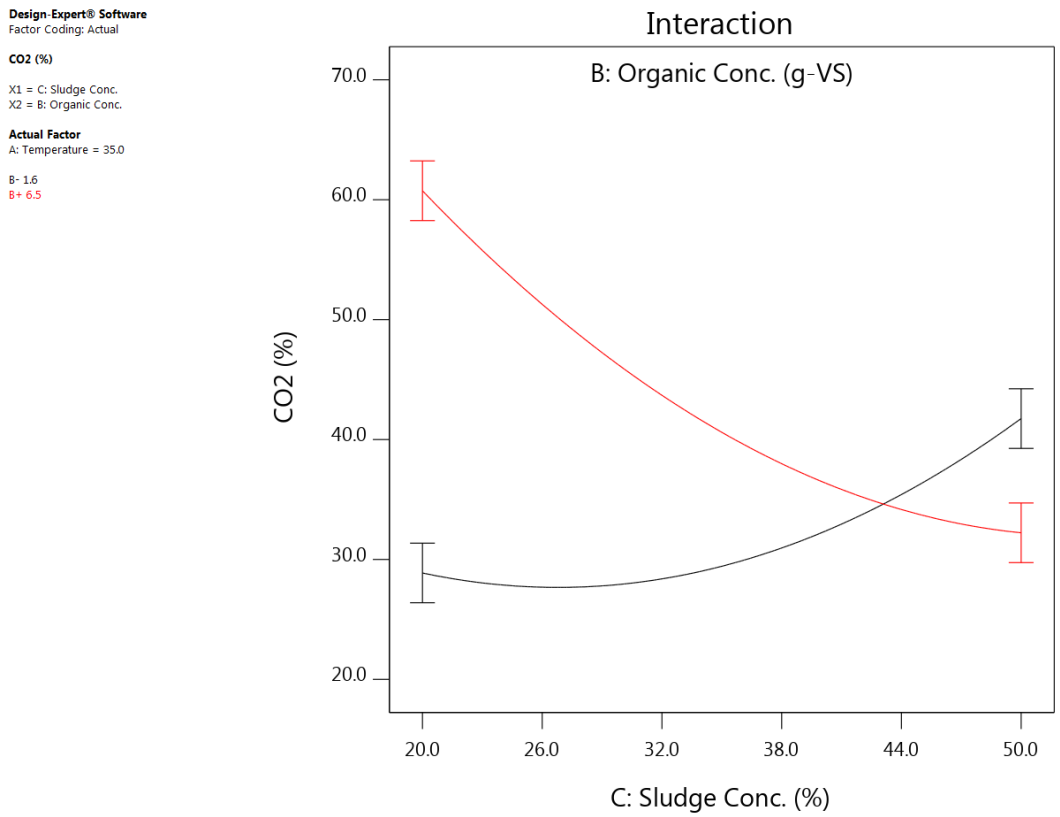


Figure 96: An interaction plot shows the influence of the interaction of the organic concentration and sludge concentration on the carbon dioxide concentration in another view.

e. Methane Content In One-Gram VS

It is evident from the ANOVA in Table 28 that, the developed model was significant and adequately fit the data. It is also clear that, the regression of the model was good, the "Pred R^2" of 0.88 was in reasonable agreement with the "Adj R-Squared" of 0.93 and the model was able to navigate the design space. Based on the ANOVA, the model terms have significant influences on the methane content were: A, B, C and B^2. The ANOVA showed that the sludge concentration (C term) has the most significant influence on the methane content (positive influence) following by the influence of the organic concentration (negative influence). Obviously, there was no significant influences of any interaction on the methane content (see Equation 28). The perturbation plot in Figure 97 depicts the influences of all factors on the methane content. According to Table 28 and as is evident in Figure 97 that the plot of the organic concentration (B) was curved, the organic concentration has a negative quadratic influence on the methane content.

Table 28: The ANOVA table for the methane content response of the AD of mango residues.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	229412.39	4	57353.10	58.007	9.48E-08	significant
A-Temperature, °C	18595.56	1	18595.56	18.808	0.000967	
B-Organic Conc., g-VS	83763.25	1	83763.25	84.718	8.71E-07	
C-Sludge Conc., %	114936.15	1	114936.15	116.246	1.58E-07	
B^2	12117.44	1	12117.44	12.256	0.004375	
Residual	11864.77	12	988.73			
Lack of Fit	11437.26	8	1429.66	13.377	0.012072	not significant
Pure Error	427.51	4	106.88			
Cor Total	241277.16	16				
R^2= 0.95				Pred R^2= 0.88		
Adj R^2= 0.93				Adeq. Precision= 26.06		

$$\text{Methane content}, \frac{cc}{g} - VS = -498.784 + 16.07083 * (\text{Temperature}, °C) + 30.41462 * (\text{Organic Conc.}, g - VS) + 7.990833 * (\text{Sludge Conc.}, \%) - 8.9111 * (\text{Organic Conc.}, g - VS)^2 \quad \text{Eqn. 28}$$

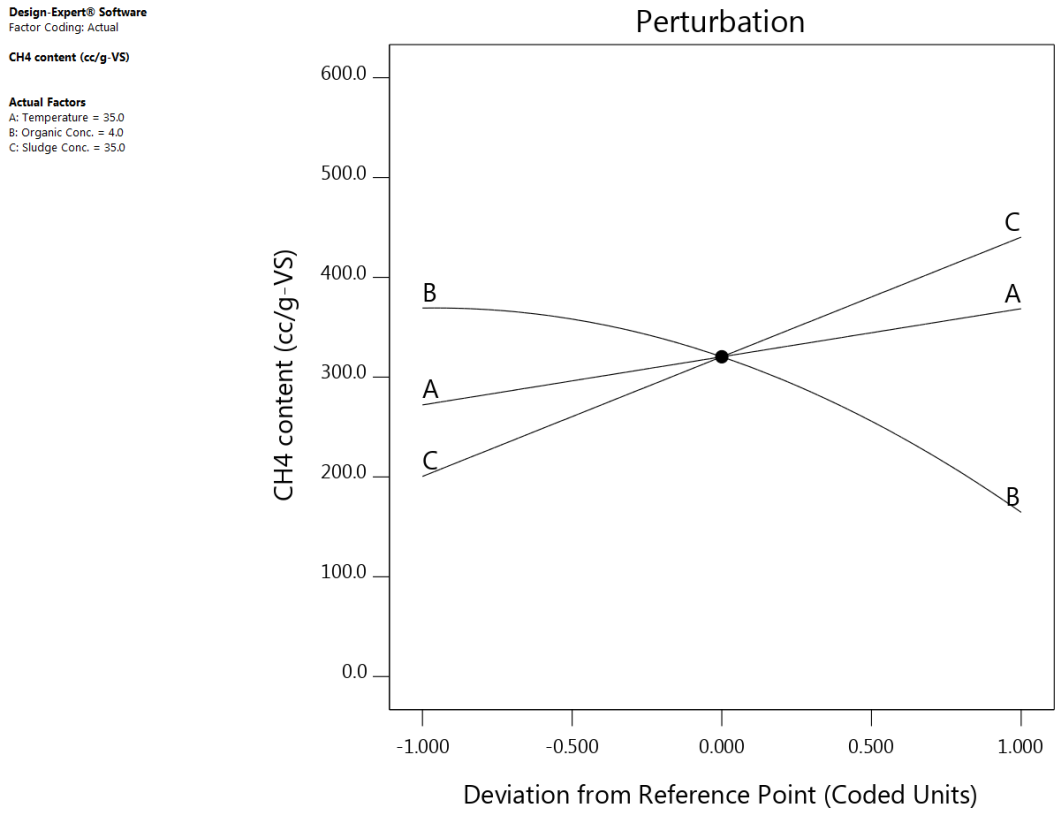


Figure 97: The Perturbation plot of the methane content response.

2. Discussion Of The Key Findings

As mango contains some important nutrients and special features such as flavours; mango is considered one of the most consumed fruits worldwide. The present study discovered that, mango residues constituted approximately one third of the total weight of the mango. This finding is in line with O'Shea, N. et al. [128] who revealed that, 35-60% of the total mango weight are discarded after processing. These discarded residues represented the main by-products of the mango fruit. According to Tesfaye, T. [133], the exploitation of the mango fruit by-products in industries, etc. helps in reducing the environmental pollution, creating job opportunities and minimising the cost of waste disposal for processing industries. Tesfaye [133] also concluded that, these by-products could be used as a potential source for starch, tannin, phenolic compound, crude fat, and protein. Therefore, the exploitation of these by-products in the production of high-value products and other bio-products competitive to their counterparts has a great benefit to the industrial sectors and the environment as well.

In terms of the preparation of the starch and the seed coats, the separation and the treatment of them did not require high electric energy ($\leq$ 0.08 kWh per processing the starch and fibres from one mango), long processing times ($\leq$ 3 hrs) or even complicated processes. Hence, their uses in producing bio-products such as bio-plastics after the AD process may help in increasing the economic feasibility of the AD plants. In addition, the results have revealed that, the total weight of the mango seed and seed coat range from 12 to 15% of the total weight of the mango, in accordance with Wu, J.S.B. et al. [51]. Approximately an 80% reduction in the seed coats weight was noticed after treating and processing them for use as reinforcement materials. The resulted starch weight of the seeds was quite close to the starch weight of the Mangifira Indica mango seeds (21%) [135].

On the other hand, a reduction in the production of the biogas was observed in the second collection. However, this reduction was lower compared to the reduction that was observed during the 2nd collection of the biogas produced from potato residues. Another observation that can be drawn from the results is that, the sludge concentration and organic concentration have significant influences on all responses. In addition, the influences of their interaction on the pH and quality of the biogas produced were significant. This influence of the interaction was similar

to some extent to its influences on the pH and the quality of the biogas produced from potato residues but in different levels. In comparison between the results of the AD of potato and mango residues in terms of the highest biogas volume produced from the one gram of the VS and the highest methane concentration, it was clear that the highest biogas volume and the highest methane concentration produced from mango residues were lower by approximately 30% and 0.5% respectively.

Due to the stiffness of the seed coats, they were not easily accessible by the hydrolytic enzymes and therefore, they were not digested by the microorganism within the control digesters. The remarkable differences between the volume of the biogas produced from the controls and the predicted volume at the same conditions of the controls clearly explain, how negatively the coats influenced the quantity of the biogas. While, the impacts of the coats on the quality of the biogas were relatively low, it is possible to say that the beating pre-treatment of the mango seed coats was not sufficient to increase the accessible surface area and size of pores available for the hydrolytic enzymes and further investigation is required to find out the appropriate pre-treatment method. This finding could also enhance the use of mango seed coats in waste-to-energy plants or in other applications i.e. industrial and commercial.

4.2.3 AD Of Avocado Residues

Post peeling the avocados, the peels were washed thoroughly with clean water and the stones were removed from the flesh. The total weight of the avocado was found ranging from 203-243g per avocado. Prior to the separation of starch from the seeds, the weight of the peels and seeds were measured and found ranging from 32.5 - 41.25g / avocado and 28-39.5g / avocado respectively. This indicated that, the edible parts represented approximately two thirds of the total weight of avocado. After processing and making the starch ready for use, its weight found representing approximately 8.5% of the total weight of the residues per avocado and 20% of an avocado seed. Furthermore, the same procedure used with the previous biomass was applied also to calculate the TS and VS of the avocado residues before and after the separation of starch (Figure 98 and Figure 99). Figure 100, Figure 101, Figure 102 and Figure 103 show the results obtained from the AD of the avocado residues (see also Appendix H). In order to investigate the differences between the application of

the whole residues and the residues without starch, on the biogas quantity and quality, nine control reactors containing the whole residues (including starch) were prepared. The obtained results from the controls were then compared with the predicted results of the biogas produced from the residues without starch at the same conditions. Table 29 shows the differences between the results of the controls and the predicted results at the same conditions.

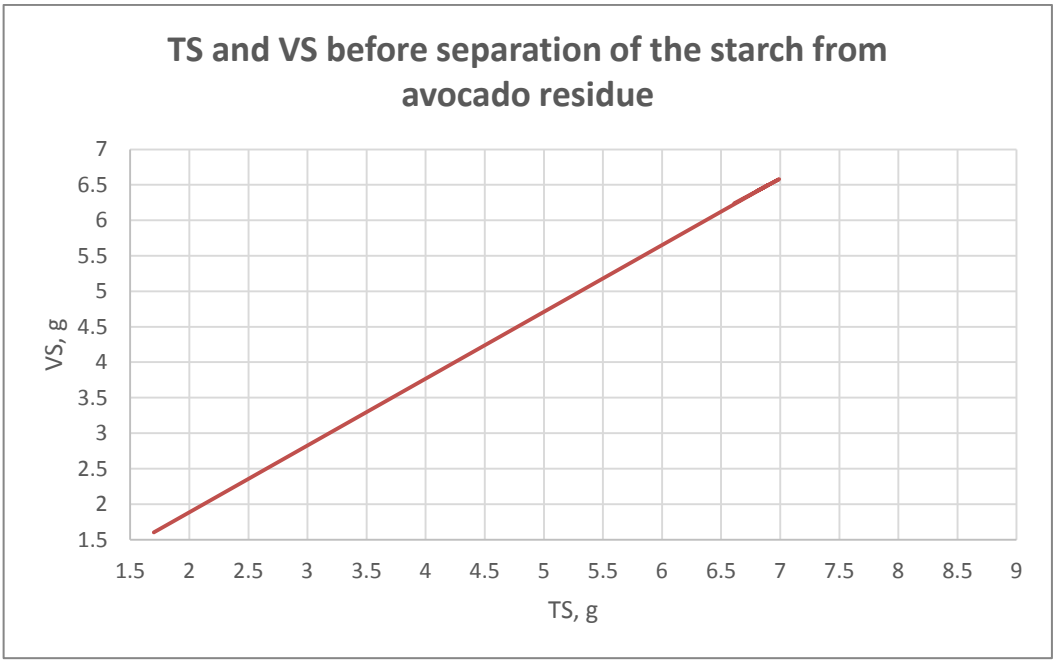


Figure 98: TS and VS before separation of the starch from avocado residue.

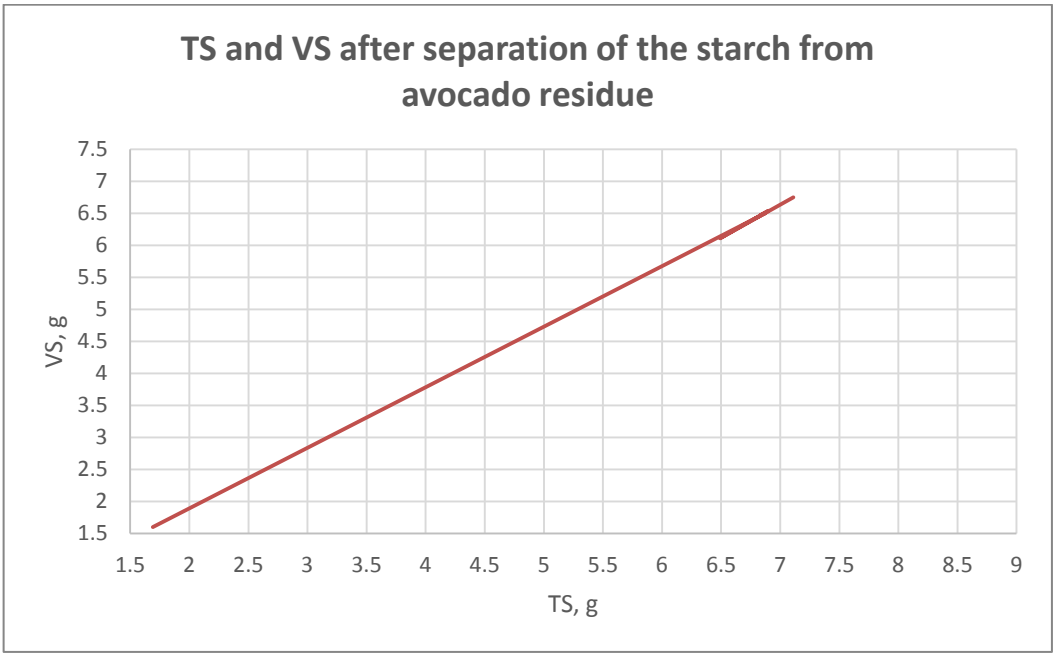


Figure 99: TS and VS after separation of the starch from avocado residue.

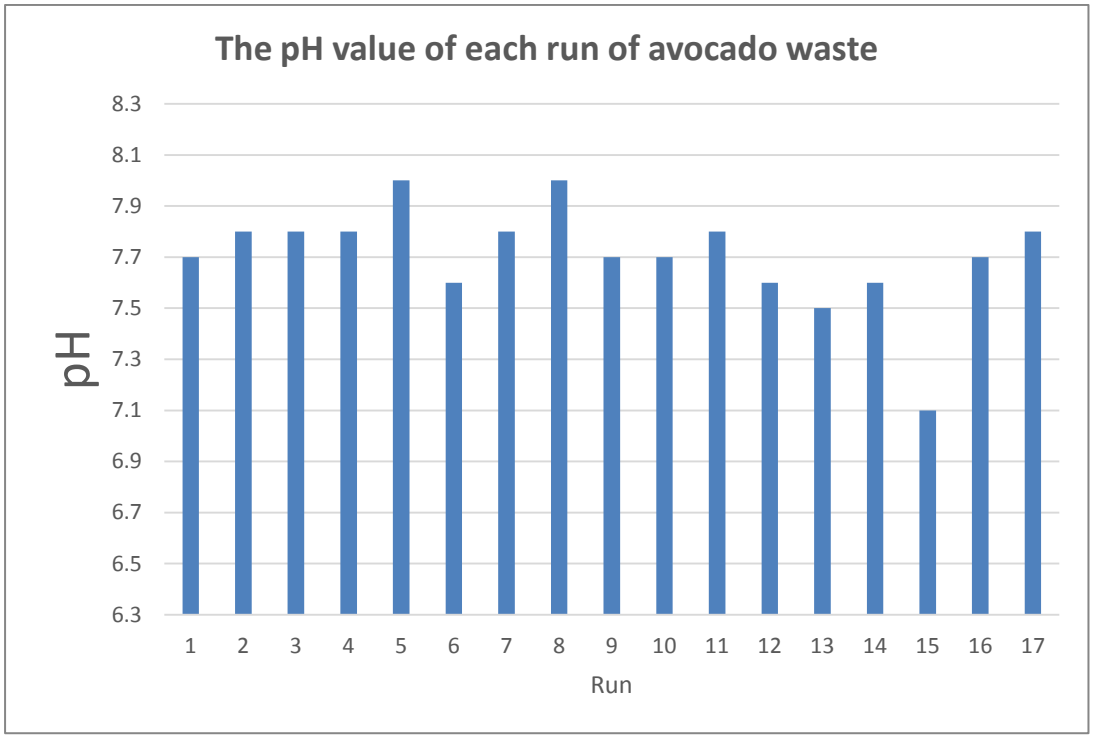


Figure 100: The pH value of each run of avocado waste.

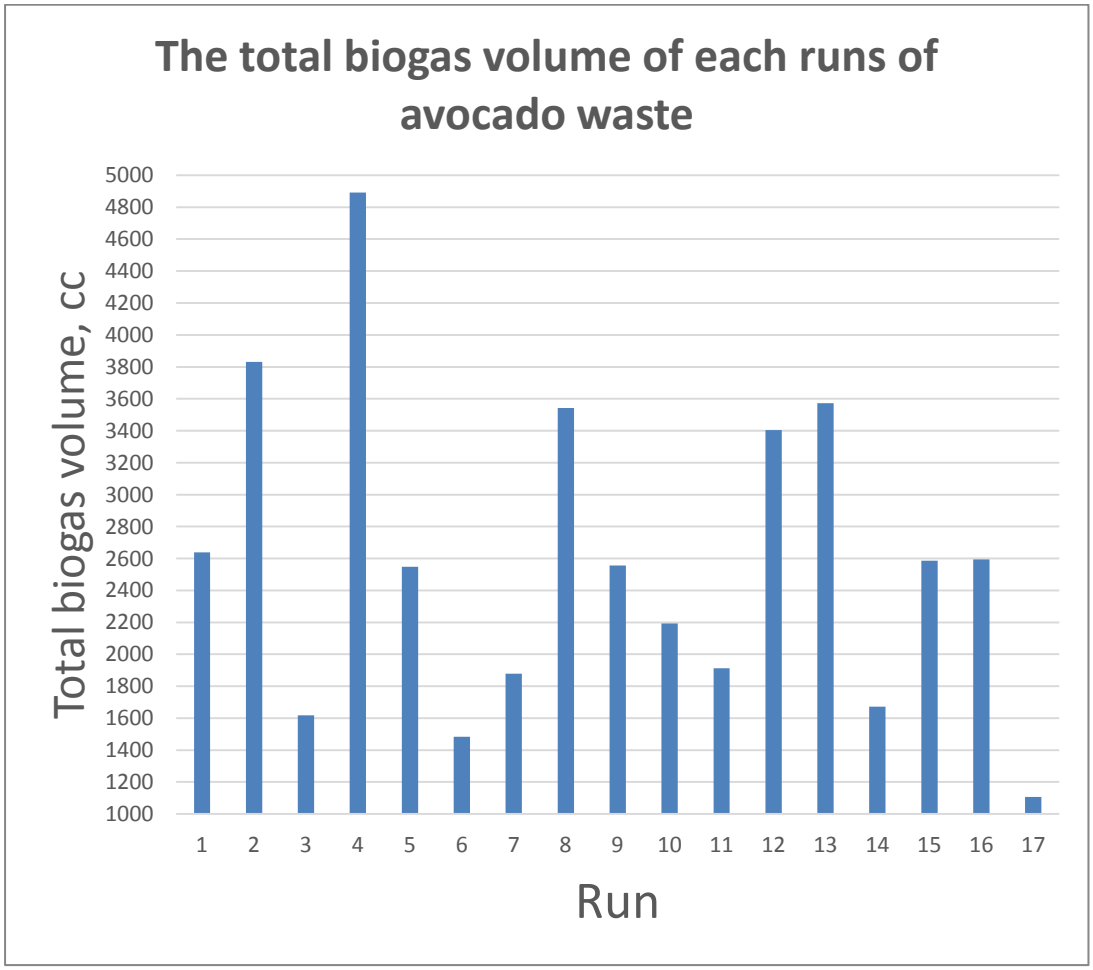


Figure 101: The total biogas volume of each runs of avocado waste.

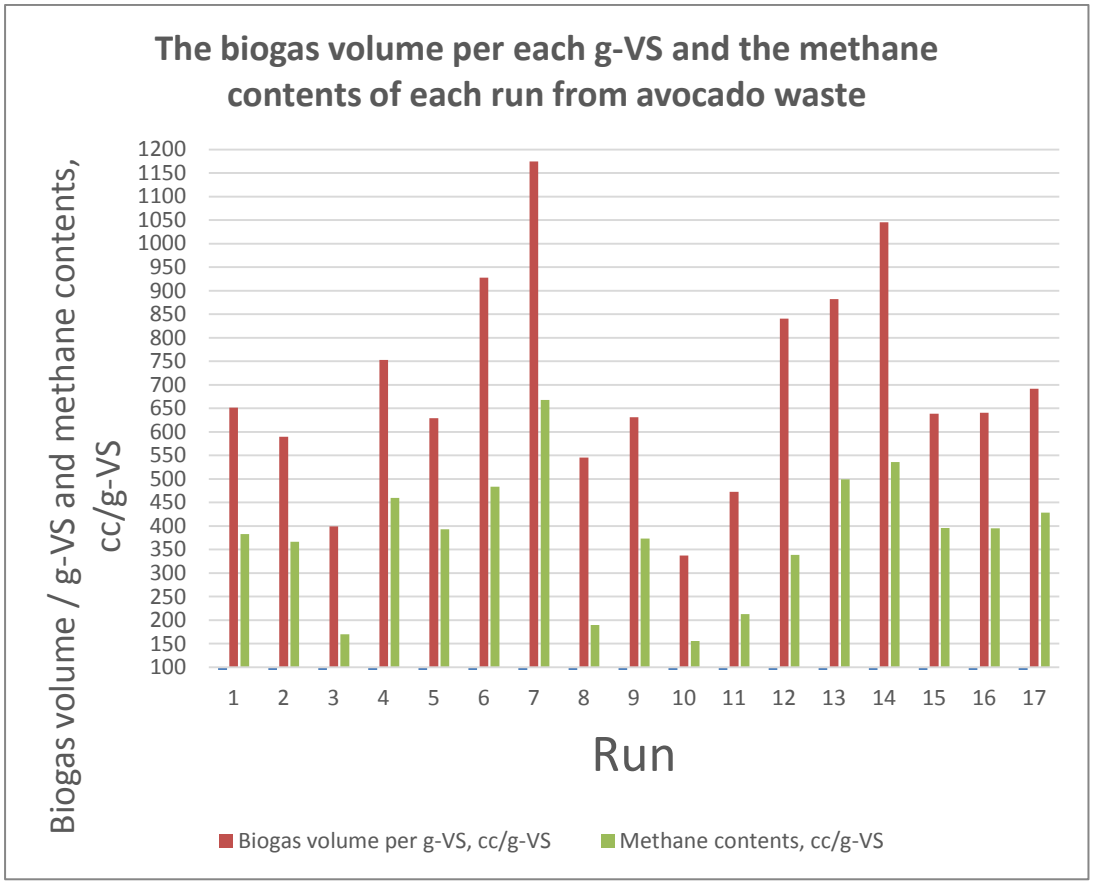


Figure 102: The biogas volume per each g-VS and the methane contents of each run from avocado waste.

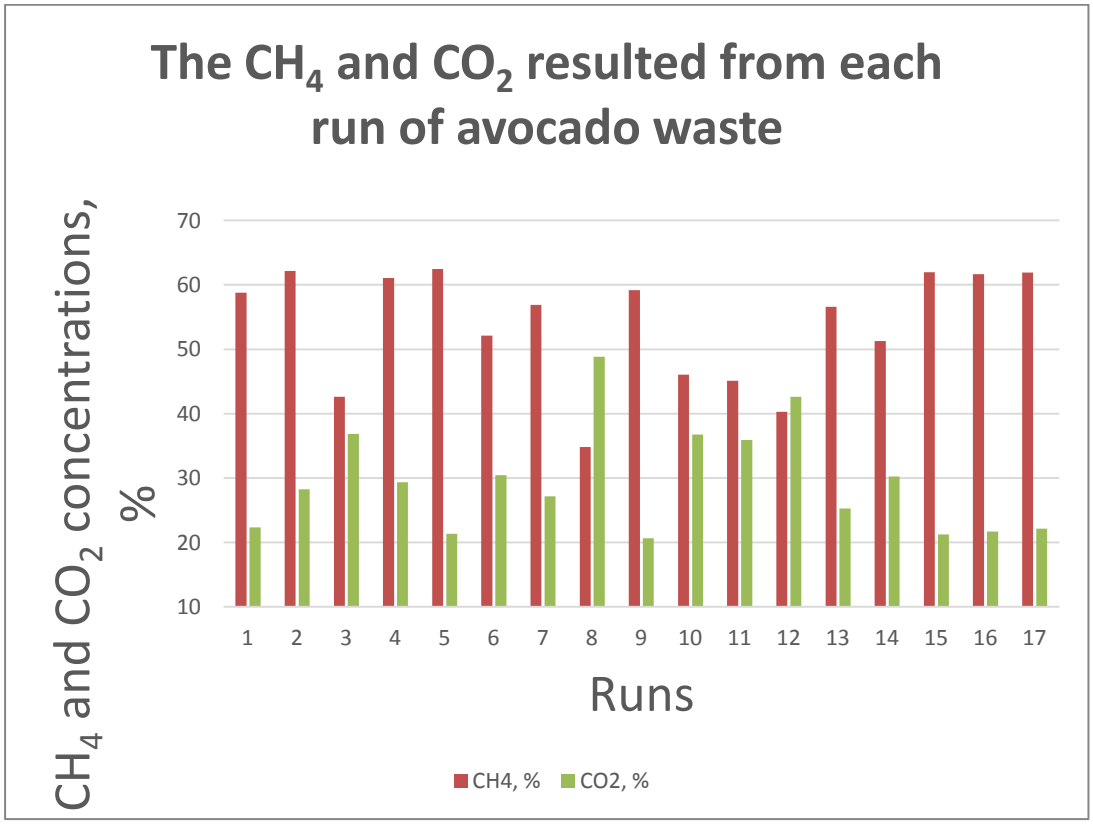


Figure 103: The CH_4 and CO_2 resulted from each run of avocado waste.

Table 29: A comparison between the results of the controls and the predicted results at same conditions.

	#	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content, cc/g-VS
Actual (controls)	1	32	6.5	50%	7.9	4802.0	738.7	42.7	46.9	315.4
	2	35			8	4965.1	763.8	62.3	32.2	475.9
	3	38			7.9	5008.3	770.4	68.7	23.1	528.9
Predicted	1	32	6.5	50%		4878.9	746.8	35.0	52.8	277.8
	2	35				4919.6	739.0	61.7	29.0	450.7
	3	38				5114.1	779.4	67.4	24.6	507.0
Difference %						-1.6	-1.1	18.0	-12.5	11.9
						0.9	3.2	0.9	9.9	5.3
						-2.1	-1.2	1.9	-6.4	4.1

According to Figure 100, the pH levels of all runs ranged from 7.1 to 8 while, the pH of the controls ranged from 7.9 to 8. This finding confirmed the equilibrium of the system and the stability of the digesters. Moreover, looking at the trend of the pH levels for all runs in Figure 100 and in Appendix H, it can be observed that, these were quite similar to the pH trends of the AD of the potato and mango residues with only slight increase in the pH for the avocado residues runs.

During the second collection of the biogas, a drop was noticed in the biogas volume compared to the first collection. This drop was lower than the drops have observed in the second collections of the potato and mango residues biogases.

In reference to Figure 102, the volumes of the resulted biogas volume produced from each g-VS for all runs ranged from 337 to 1175 cc/g-VS. In terms of the biogas quality, the methane concentration ranged from 34.8 to 62.4% while, the highest and lowest carbon dioxide concentration were 48.8% and 20.6 % which resulted for run 14 and 17 (centre points run) respectively (Figure 103).

According to Table 29, the differences between the results of the controls and the predicted ones were relatively low especially, the quantity of the biogas

produced. This can be justified by the relatively low starch weight in the controls compared to the total weight of the biomass in the other samples.

1. Analysis Of The Responses

a. Total Volume Of Biogas Produced

Table 30 is the ANOVA of the total biogas volume of the biogas produced from the AD of the avocado residues. The ANOVA confirmed that the model was significant. As is shown in the table the model terms A, B, C, BC and A^2 were significant. The analysis also revealed that the regression of the model was good as the R^2, adjusted R^2, and predicted-R^2 were all close to 1. Based on the analysis, the "Pred R-Squared" was in reasonable agreement with the "Adj R-Squared" of and the "Adeq Precision" was greater than 4. Therefore, it can be said that the model was adequately fit the data and able to be used to navigate the design space. Moreover, the model term B had the largest significant influence on the total volume of the biogas, following by the sludge concentration (C term), BC and A respectively. Equation 29 shows the final equation in terms of actual values for this response as was computed by the software.

Table 30: The ANOVA table for the total biogas volume response of the AD of avocado residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	15694880	6	2615813.35	1489.09	3.66E-14	significant
A-Temperature, °C	110623.3	1	110623.31	62.97	1.26E-05	
B-Organic Conc., g-VS	8647347	1	8647346.88	4922.62	8.43E-15	
C-Sludge Conc., %	5979362	1	5979362.31	3403.84	5.31E-14	
BC	927754.3	1	927754.34	528.14	5.5E-10	
A^2	25014.68	1	25014.68	14.24	0.00364	
C^2	6053.992	1	6053.99	3.45	0.093059	
Residual	17566.54	10	1756.65			
Lack of Fit	12410.53	6	2068.42	1.60	0.336876	not significant
Pure Error	5156.01	4	1289.00			
Cor Total	15712447	16				
R^2= 0.99				Pred R^2= 0.99		
Adj R^2= 0.99				Adeq Precision= 141.60		

$$\text{Total Biogas, cc} = 9591.873 - 559.466 * (\text{Temperature, °C}) - 34.3107 * (\text{Organic Conc., g} - \text{VS}) + 16.34192 * (\text{Sludge Conc., \%}) + 13.10476 * (\text{Organic Conc., g} - \text{VS}) * (\text{Sludge Conc., \%}) + 8.552329 * (\text{Temperature, °C})^2 - 0.16829 * ((\text{Sludge Conc., \%})^2 \quad \text{Eqn. 29}$$

The normal residual plot in Figure 104 revealed that the residuals were normally distributed. On the other hand, the predicted values versus the actual values plot (Figure 105) indicated that, there was a good correlation between the predicted and the actual results of the model.

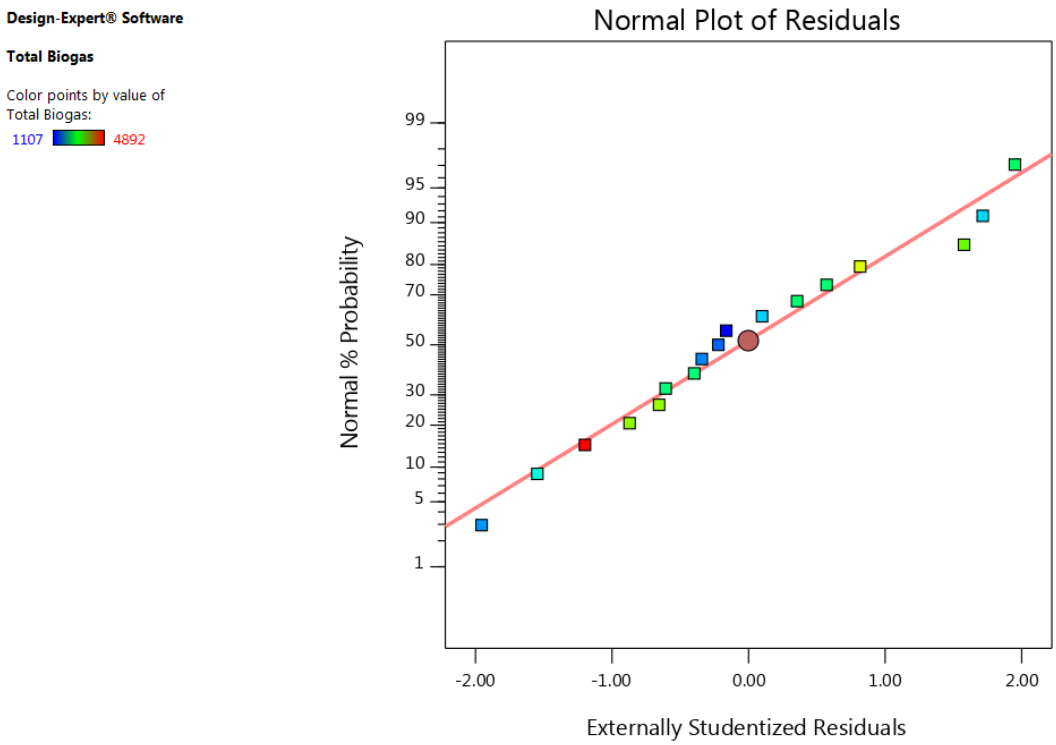


Figure 104: The normal plot of residuals.

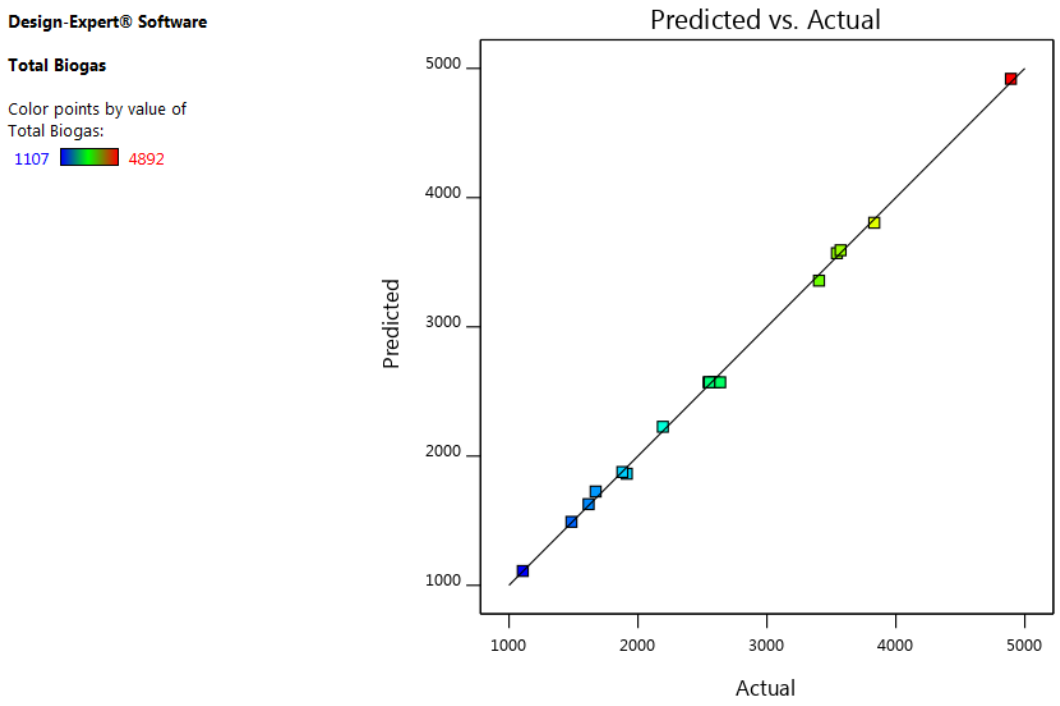


Figure 105: The scatter plot of the predicted values versus the actual values.

Figure 106 shows that an increase of any factor of the process was accompanied by an increase in the total biogas volume. The extent of the increase in the total volume did vary from one factor to another. As is shown in Figure 106, an increase in the temperature (A) resulted in a slight increase in the total volume. On the contrary, an increase in the organic concentration led to a large increase in the total volume.

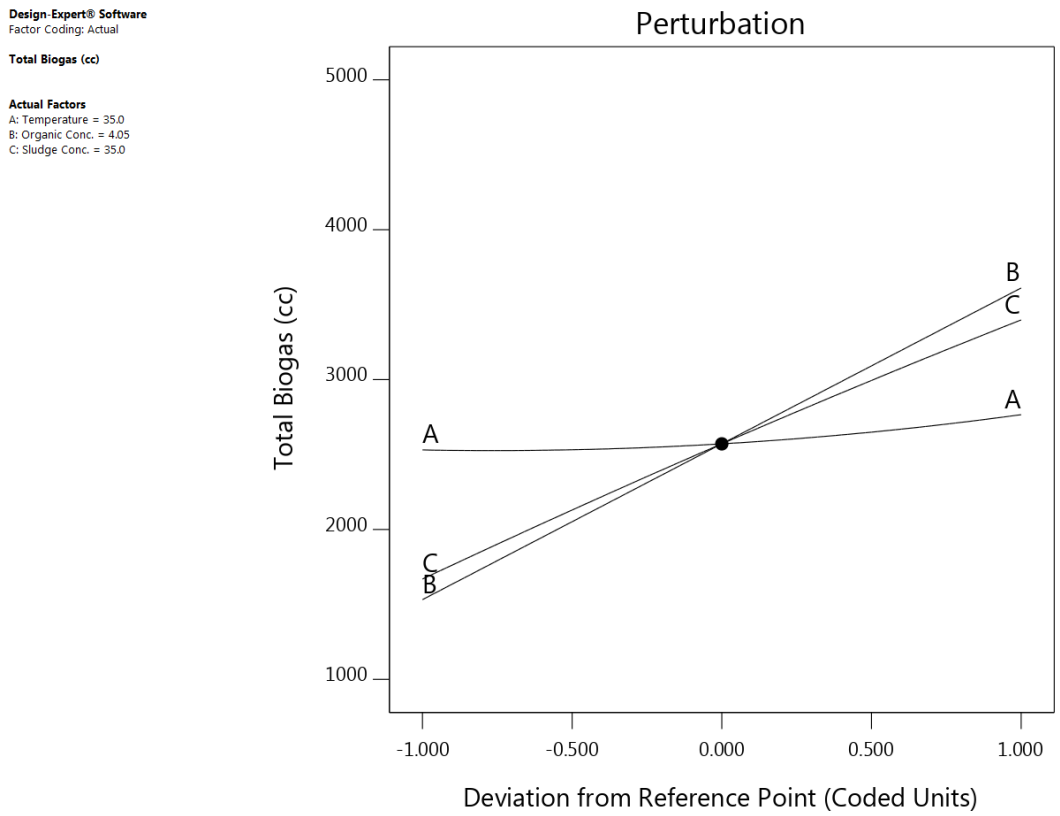


Figure 106: The Perturbation plot of the total biogas volume response.

Figure 107 and Figure 108 illustrate the influence of the interaction (BC) of the organic concentration and the sludge concentration on the total volume of the biogas produced. As the temperature has the lowest significant influence on this response, Figure 107 and Figure 108 are illustrating this interaction at the mid-level of temperature (35 °C). It can be noted that, and 1.6 g-VS, the influence of the interaction was slightly significant. The significance of the influence increased as the organic concentration and sludge concentration increased. When the organic concentration was set to its highest level, a large variation in the biogas volume was observed when the sludge concentration is increased or decreased. Figure 109 also shows the influence of the interaction but in a 3D surface plot.

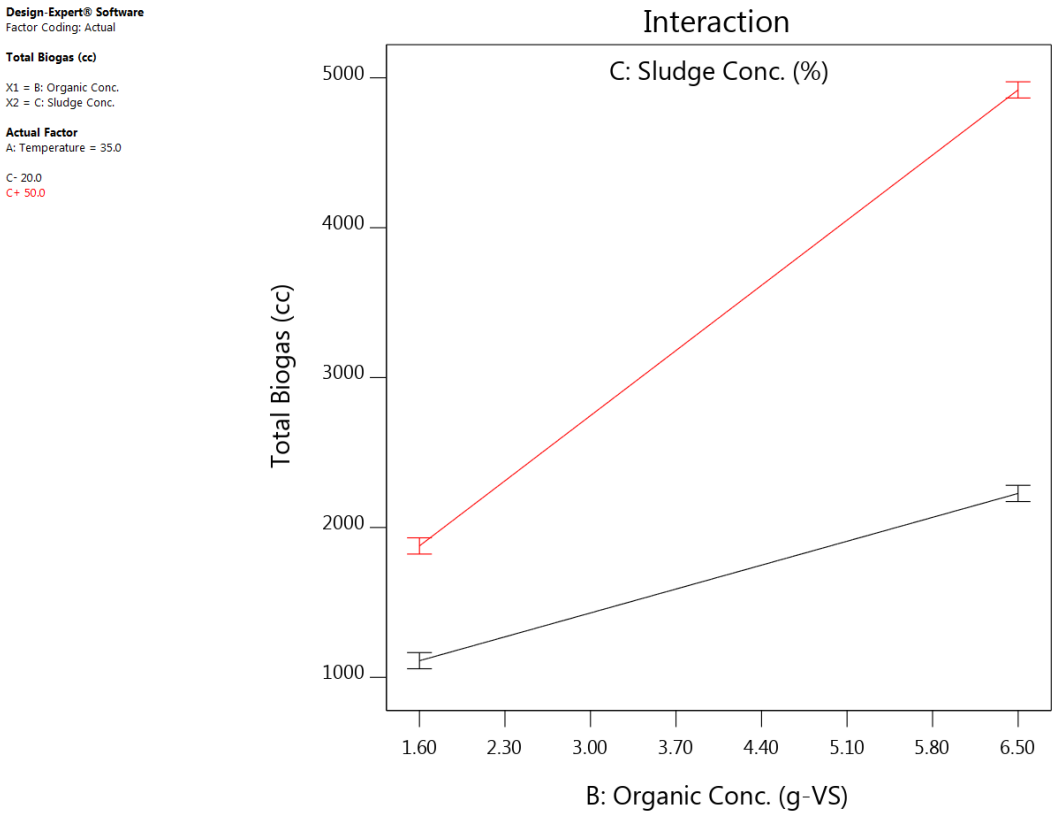


Figure 107: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the total volume of the biogas.

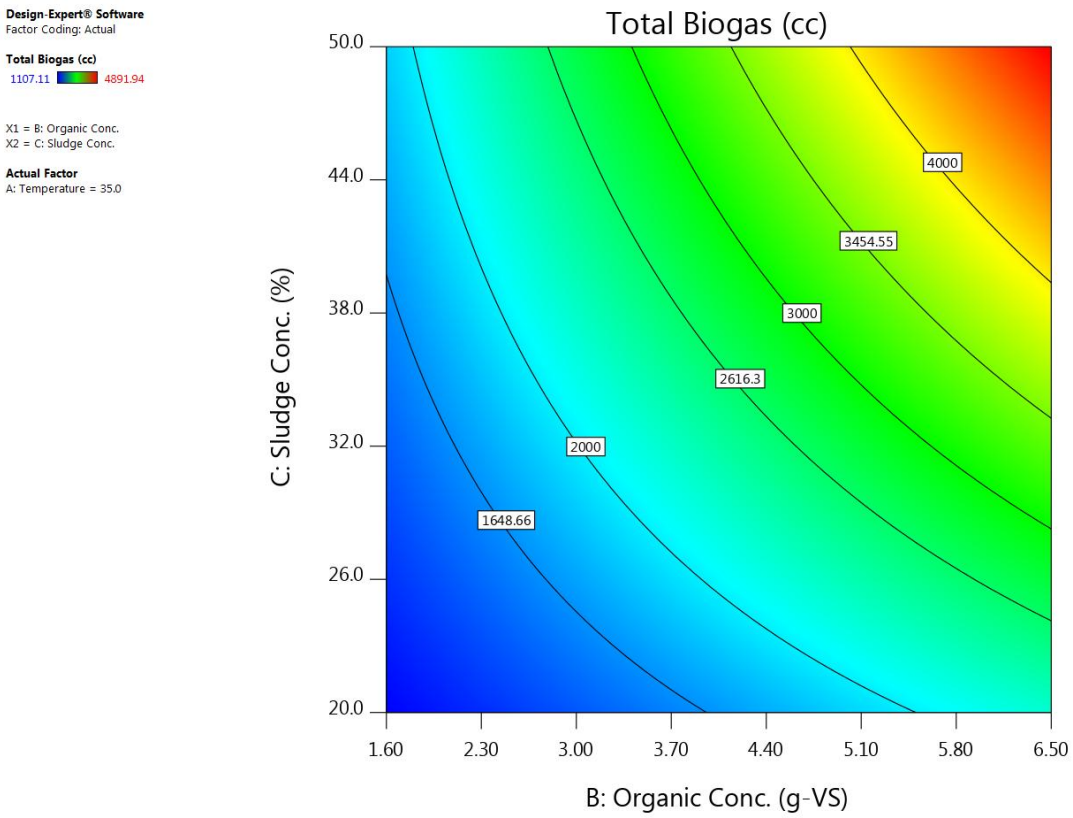


Figure 108: A contour plot shows the influence of the organic concentration and sludge concentration interaction on the total biogas volume.

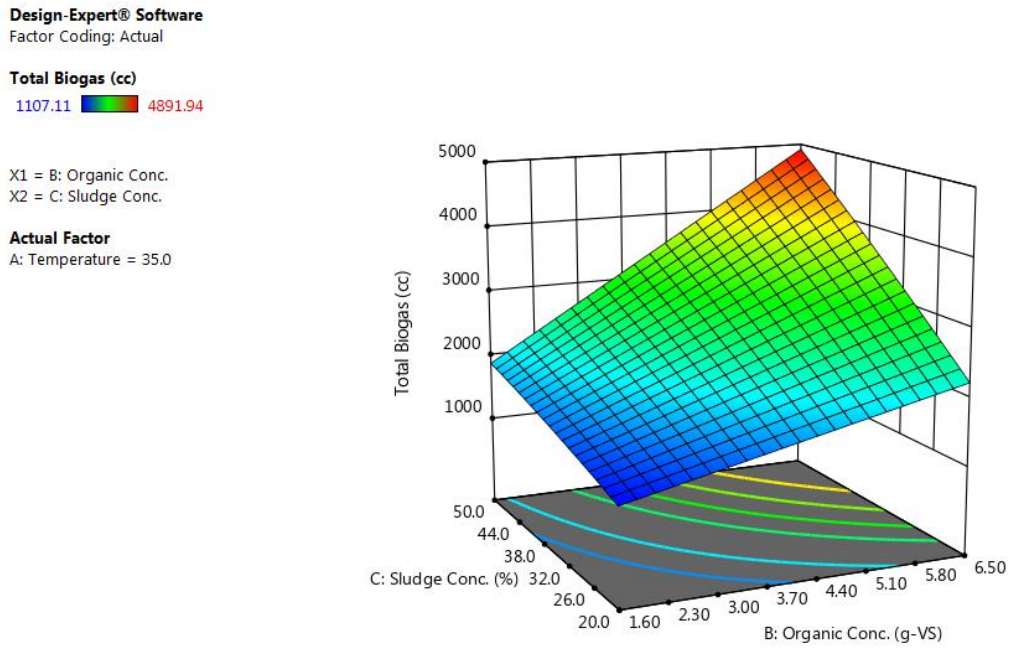


Figure 109: A 3D surface plot shows the influence of the organic concentration and sludge concentration interaction on the total biogas volume.

b. Volume Of Biogas Produced From Each Gram VS

The ANOVA analysis of the response tested the adequacy of the developed model and found it significant and adequately fit the data. Also, the analysis proved that the regression of the model was good, the "Pred R-Squared" of 0.98 was in reasonable agreement with the "Adj R-Squared" of 0.99 and the model was able to navigate the design space. According to Table 31, the model terms have significant influences on the biogas volume produced from the gram of the VS were: A, B, C, AB, BC, A^2 and B^2. The ANOVA also revealed that, the sludge concentration (C) had the most significant influence on the response while, the organic concentration (B) had the second most significant influence. Equation 30 shows the mathematical model of this response in actual values.

Table 31: The ANOVA table for the biogas volume produced from the g-VS of the AD of avocado residues.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	779391.8	8	97423.97	487.437	6.12E-10	significant
A-Temperature, °C	9555.02	1	9555.02	47.806	0.000123	
B-Organic Conc., g-VS	325849.8	1	325849.8	1630.31	1.56E-10	
C-Sludge Conc., %	382191.5	1	382191.55	1912.20	8.25E-11	
AB	1334.47	1	1334.47	6.68	0.032417	
BC	1140.39	1	1140.39	5.71	0.043948	
A^2	2432.28	1	2432.28	12.17	0.008217	
B^2	55355.86	1	55355.86	276.96	1.72E-07	
C^2	778.47	1	778.47	3.89	0.083883	
Residual	1598.96	8	199.87			
Lack of Fit	1284.61	4	321.15	4.09	0.10075	not significant
Pure Error	314.34	4	78.59			
Cor Total	780990.73	16				
R^2= 0.99				Pred R^2= 0.98		
Adj R^2= 0.99				Adeq Precision= 81.73		

$$\text{Biogas volume per 1g of VS, CC } (\text{g} - \text{VS})^{-1} = 3151.8141 - 165.35205 * (\text{Temperature, °C}) - 134.04444 * (\text{Organic Conc., g} - \text{VS}) + 20.662542\ (\text{Sludge Conc., \%}) - 2.4850653 * (\text{Temperature, °C}) * (\text{Organic Conc., g} - \text{VS}) - 0.4594522 * (\text{Organic Conc., g} - \text{VS}) * (\text{Sludge Conc., \%}) + 2.6705213 * (\text{Temperature, °C})^2 + 19.102114 * (\text{Organic Conc., g} - \text{VS})^2 - 0.0604323 * (\text{Sludge Conc., \%})^2 \quad \text{Eqn. 30}$$

Figure 110 is the normal residual plot of the response. It shows that the residuals were normally distributed. The predicted values versus the actual values plot (Figure 111) is the results of the test of checking for constant errors. From the plot, it is evident that there was a good correlation between the predicted and the actual results of the model.

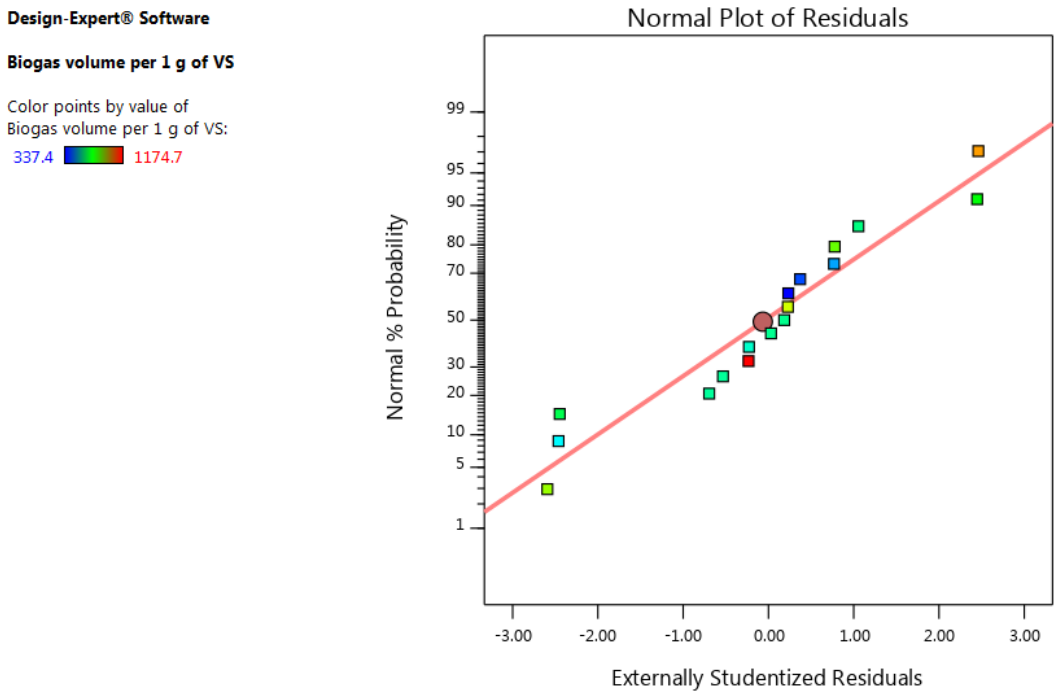


Figure 110: The normal plot of residuals.

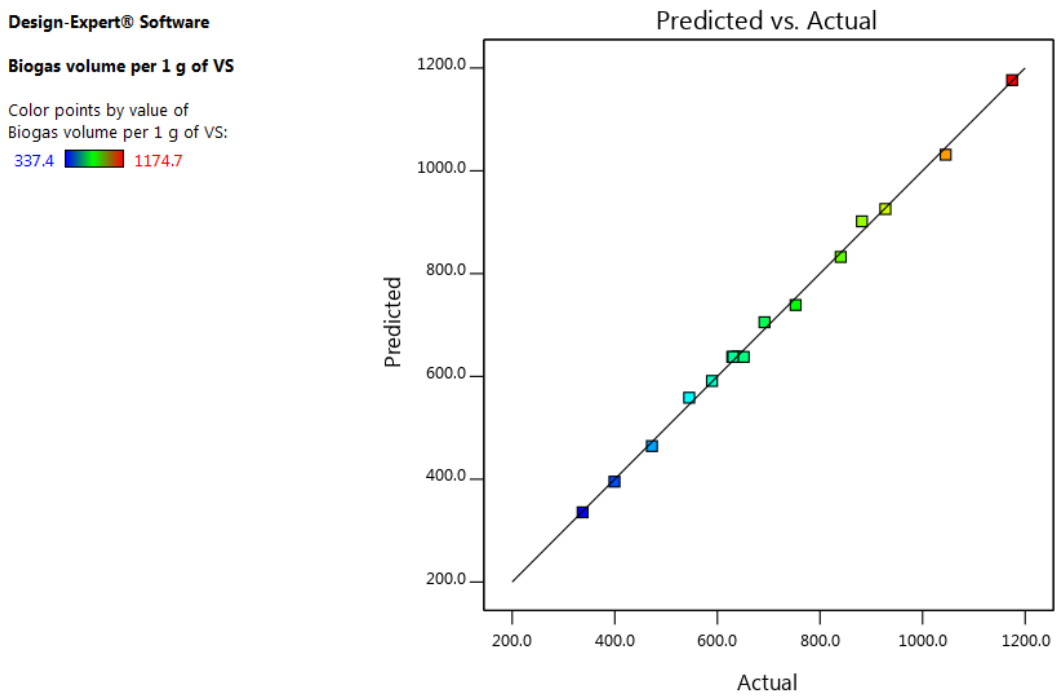


Figure 111: The scatter plot of the predicted values versus the actual values.

According to Figure 112, the biogas volume produced from each gram VS slightly increased as the temperature increased. In contrary, it increased sharply with the increases of the sludge concentration. As is clear, the organic concentration had an opposite and lower influence on this response than the influence of the sludge concentration.

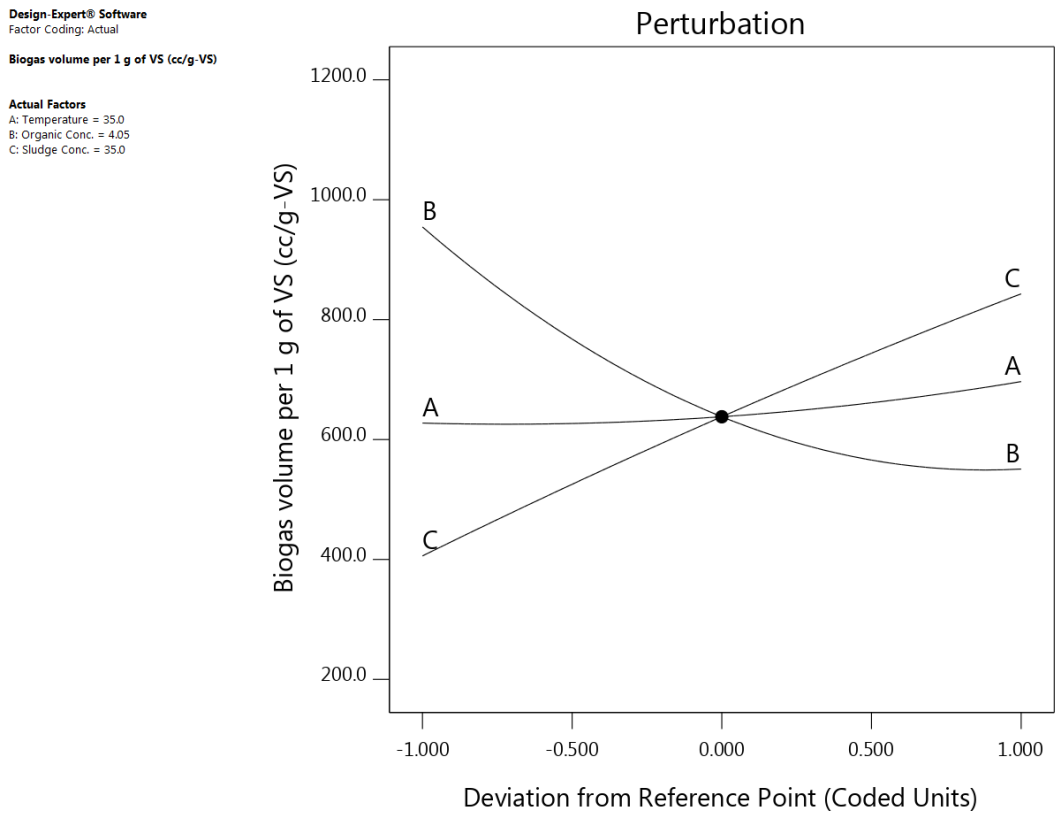


Figure 112: The Perturbation plot of the biogas volume produced from each gram VS.

Figure 113 and Figure 114 are interaction plots illustrate the influence of the AB and BC interactions on the response. As is shown in Figure 113, the influence of AB was insignificant when the organic concentration was set to its highest level while, it was slightly significant when it was set to its lowest level. Figure 114 revealed that, the significance of the influence of the BC was almost the same on the studied range with a slight increase in the significance when the sludge concentration was set to its highest level.

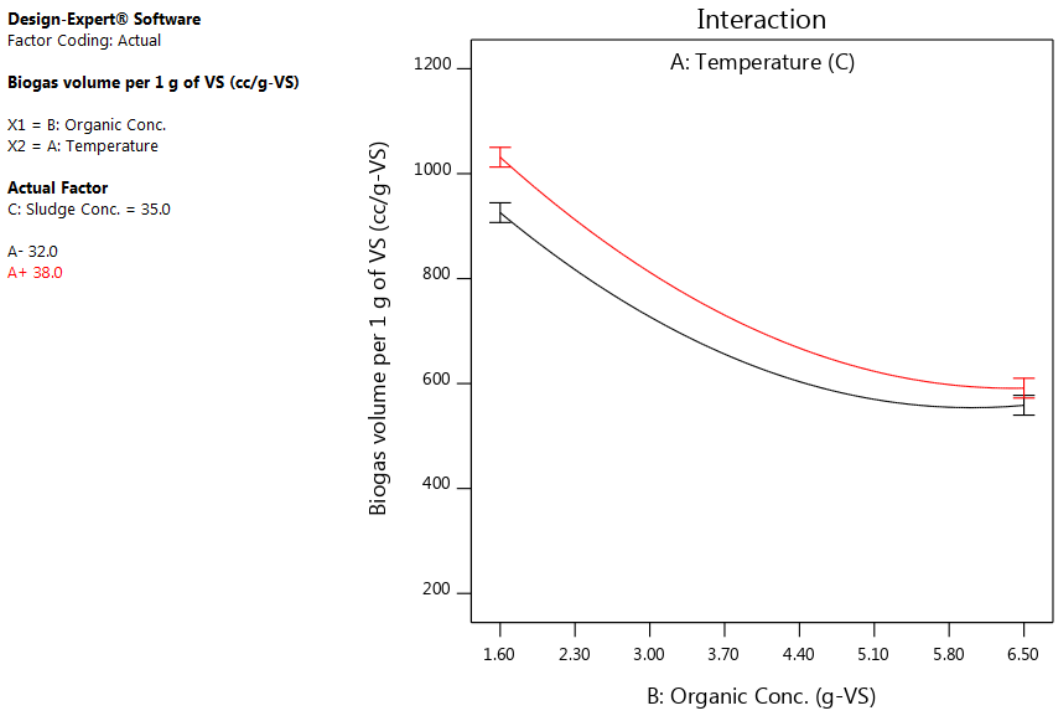


Figure 113: An interaction plot shows the influence of the interaction of the temperature and organic concentration on the biogas volume produced from each gram VS.

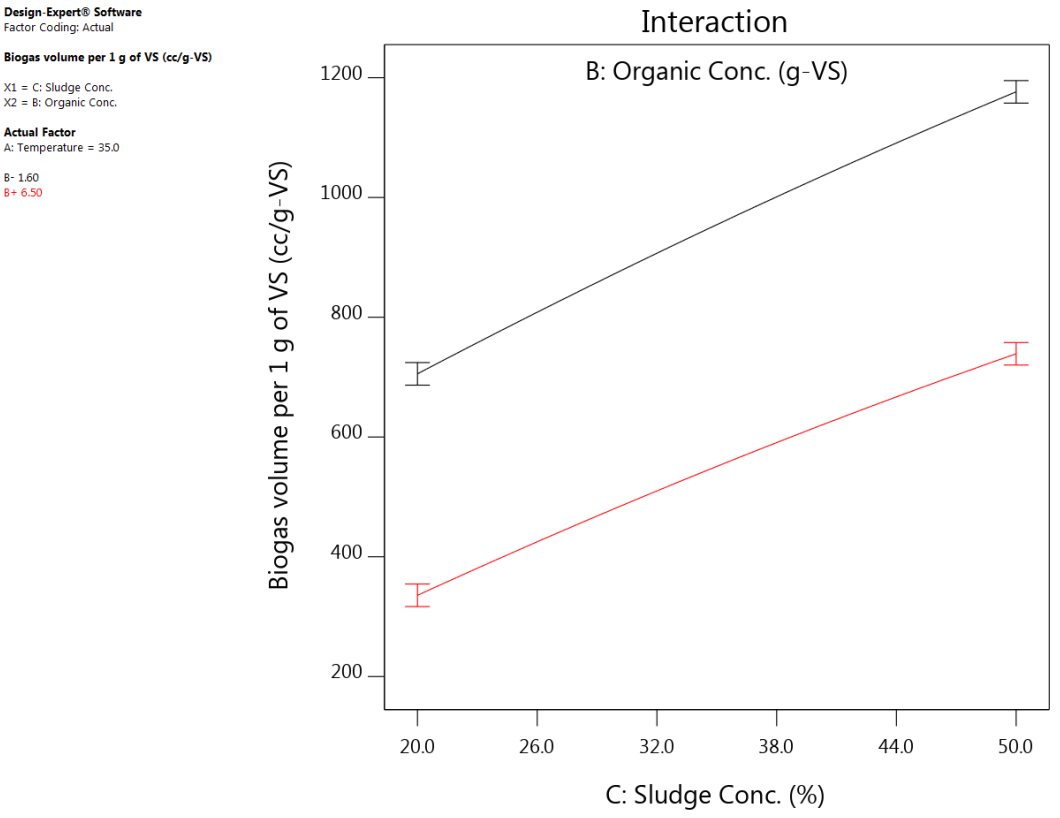


Figure 114: An interaction plot shows the influence of the interaction of the organic concentration and sludge concentration on the biogas volume produced from each gram VS.

c. Methane Concentration

The ANOVA of the methane concentration confirmed the following: the developed model was significant and adequately fit the data, the regression of the model was good and the model was able to navigate the design space (see Table 32). The analysis also showed that, the following model terms have significant influences on the methane concentration: A, B, C, AB, AC, BC, A^2 and C^2. It was also confirmed that, the model term A^2 has the most significant influence on the response, following by the temperature AB and A respectively. Equation 31 shows the final equation in terms of actual factors for this response as was computed by the software.

Table 32: The ANOVA table for the methane concentration of the AD of avocado residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1253.016	8	156.6271	55.439	3.3E-06	significant
A-Temperature, °C	256.5113	1	256.5113	90.79	1.22E-05	
B-Organic Conc., g-VS	40.80056	1	40.80056	14.44	0.005235	
C-Sludge Conc., %	45.92	1	45.92	16.25	0.00378	
AB	199.28	1	199.28	70.54	3.07E-05	
AC	47.61	1	47.61	16.85	0.003414	
BC	100.33	1	100.33	35.51	0.000338	
A^2	467.88	1	467.88	165.61	1.26E-06	
C^2	72.49	1	72.49	25.66	0.000971	
Residual	22.60	8	2.83			
Lack of Fit	11.02	4	2.75	0.95	0.518673	not significant
Pure Error	11.58	4	2.90			
Cor Total	1275.62	16				
R^2= 0.98				Pred R^2= 0.90		
Adj R^2= 0.96				Adeq Precision= 21.68		

$$\text{Methane concentration}, \% = -1213.21 + 77.18964 * (\text{Temperature}, °\text{C}) - 39.3027 * (\text{Organic Conc.}, \text{g} - \text{VS}) - 1.78649 (\text{Sludge Conc.}, \%) + 0.960317 * (\text{Temperature}, °\text{C}) * (\text{Organic Conc.}, \text{g} - \text{VS}) + 0.076667 * (\text{Temperature}, °\text{C}) * (\text{Sludge Conc.}, \%) + 0.136281 * (\text{Organic Conc.}, \text{g} - \text{VS}) * (\text{Sludge Conc.}, \%) - 1.16964 * (\text{Temperature}, °\text{C})^2 - 0.01842 * (\text{Sludge Conc.}, \%)^2 \quad \text{Eqn. 31}$$

Figure 115 shows the perturbation plot of the response. Looking at the plot, a decreasing pattern can be observed in methane concentration as the organic

concentration increased. Based on the perturbation plot, as the temperature increases from its lowest level, the methane concentration increased until it is reached its peak after the reference point and then began decreased. The sludge concentration behaved almost similar to the temperature with lower significant influence. Therefore, as sludge concentration increased from the lowest level, the methane concentration increased until it is reached its peak, stayed constant for a short range and then began to decrease.

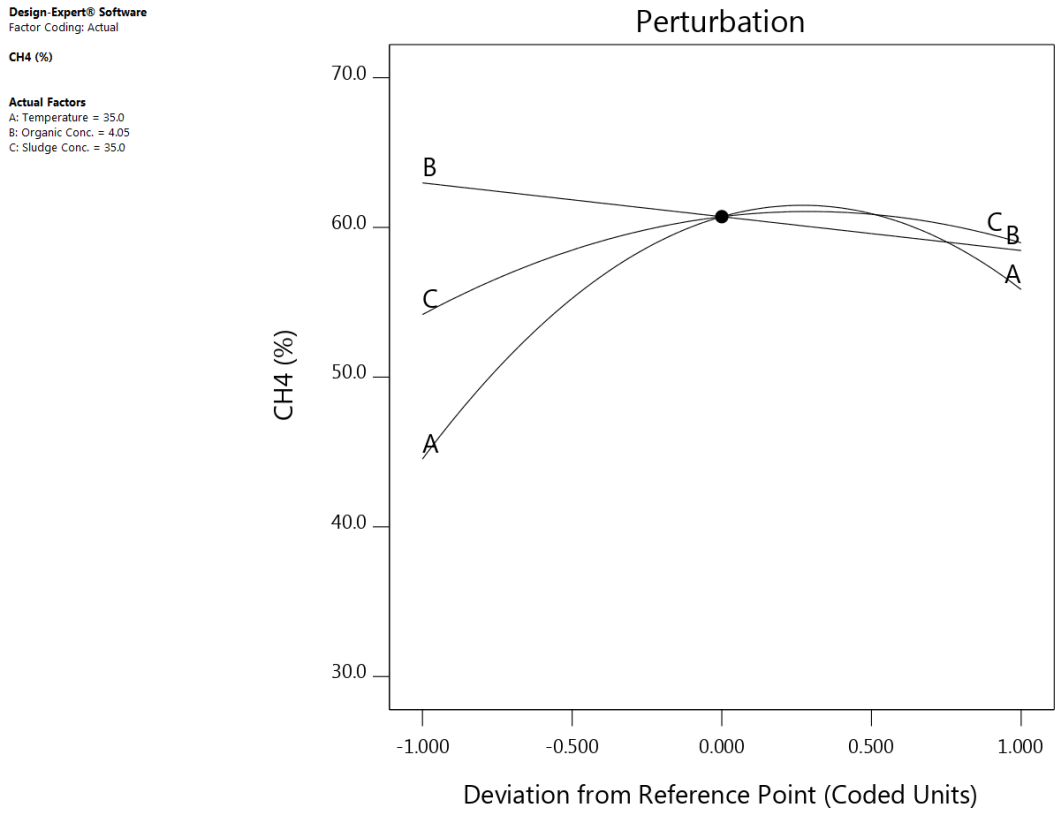


Figure 115: The Perturbation plot of the methane concentration.

Figure 116, Figure 117 and Figure 118 show the interactions of the temperature × organic concentration (AB), temperature × sludge concentration (AC) and organic concentration × sludge concentration (BC) on the methane concentration respectively. The interaction plot in Figure 116 illustrate clearly that, at the temperature= 38 °C, the changes in the concentration of the organic does not make major changes in the methane concentration. While at the temperature= 35 °C the changes was higher in the methane concentration. It is also evident that, the extent of the influence of the interaction increased as the temperature decreased. According to Figure 116, when the temperature was set to approximately 36 °C and sludge concentration fixed at 35%, the methane concentration was constant and the

changes in the organic concentration did not make any changes. On the other hand, Figure 117 illustrates that, when the organic concentration was set equal to 4.05 g-VS, the influence of the AC interaction was insignificant when the temperature was set to its lowest level, while it was significant when the temperature was set to its highest level. As is clear from the plot of the AC interaction, there was an overlapping in the sludge concentrations when the temperature was set equal to 33 °C. This means that, the influence of the interaction was insignificant at the temperature of 33 °C. Figure 118 shows that, when the temperature (A) was set to its mid level, the influence of the interaction of the organic concentration and sludge concentration was slightly significant when the organic concentration was set to its lowest level, while it was significant when the organic concentration was set to its highest level. As is evident from the plot, there was an overlapping in the sludge concentrations when the organic concentration was closer to 3 g-VS.

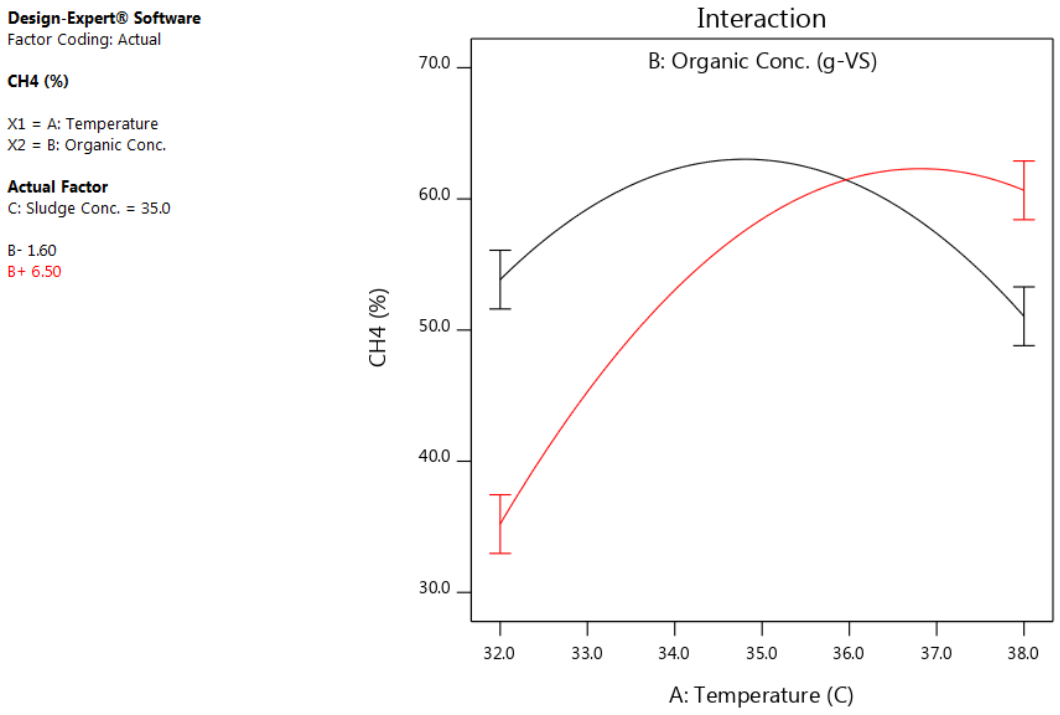


Figure 116: An interaction plot shows the influence of the temperature and organic concentration interaction on the methane concentration.

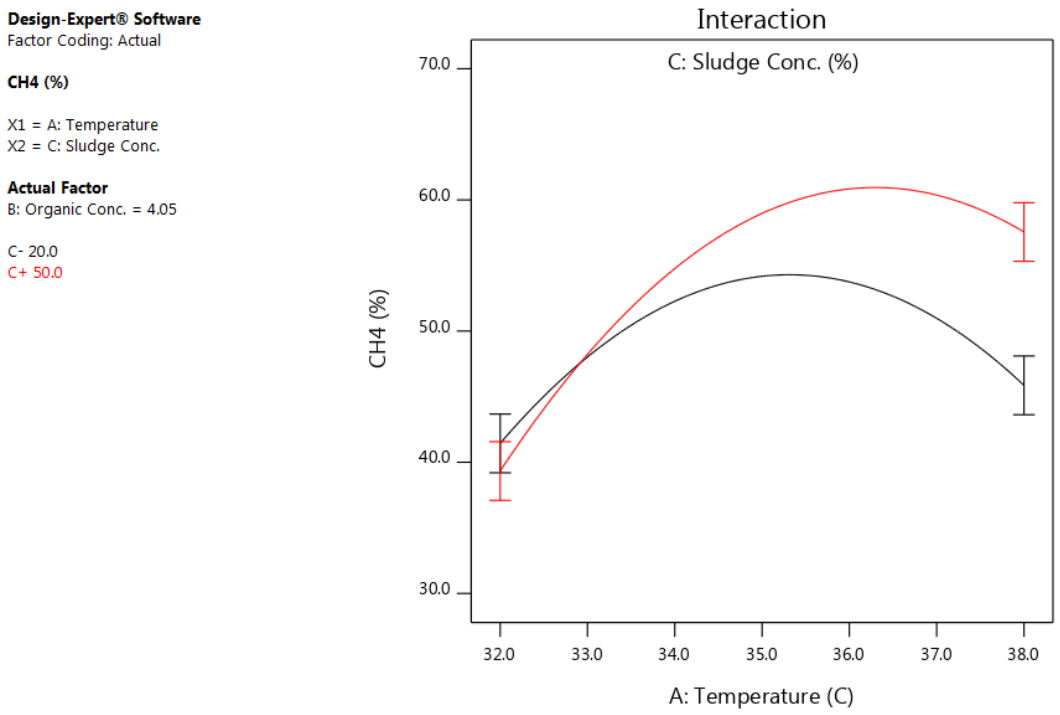


Figure 117: An interaction plot shows the influence of the temperature and sludge concentration interaction on the methane concentration.

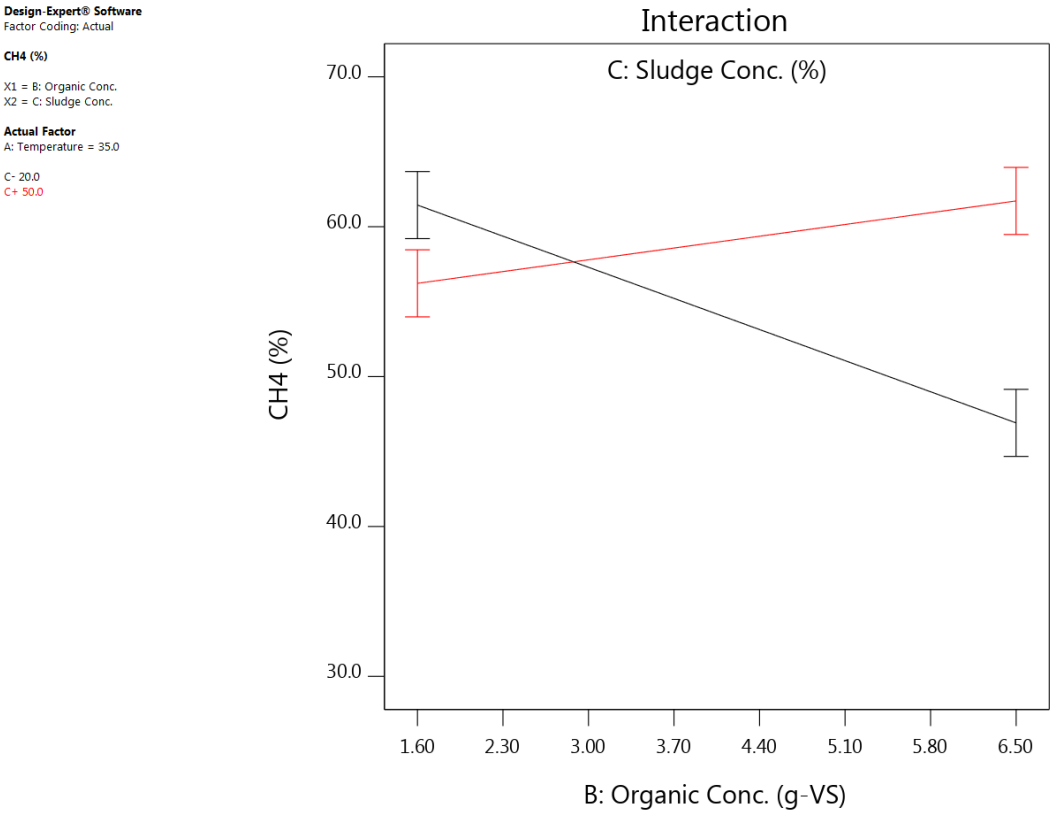


Figure 118: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the methane concentration.

d. Carbon Dioxide Concentration

The ANOVA table (Table 33) confirmed that the developed model was significant, adequately fit the data, its regression was good and it can be used to navigate the design space. In addition to the significant influence of the B^2, the carbon dioxide concentration response was influenced significantly by the same terms influenced methane concentration response but, in different amounts. The ANOVA also shows that, the model term (A^2) has the largest significant influence on the carbon dioxide concentration, following by the AB and the temperature (A) (same as the methane concentration response). Equation 32 illustrates the computed final equation of this response in terms of actual values.

Table 33: The ANOVA table for the carbon dioxide concentration of the AD of avocado residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1097.107	9	121.900	274.257	4.44563E-08	Significant
A-Temperature, °C	190.125	1	190.125	427.750	1.55122E-07	
B-Organic Conc., g	137.78	1	137.78	309.982	4.69802E-07	
C-Sludge Conc., %	6.60	1	6.60	14.85	0.006263217	
AB	104.04	1	104.04	234.07	1.22806E-06	
AC	67.79	1	67.79	152.51	5.24269E-06	
BC	38.44	1	38.44	86.48	3.44556E-05	
A^2	392.23	1	392.23	882.45	1.26234E-08	
B^2	47.30	1	47.30	106.42	1.74225E-05	
C^2	69.69	1	69.69	156.79	4.77652E-06	
Residual	3.11	7	0.44			
Lack of Fit	1.54	3	0.51	1.31	0.387498486	not significant
Pure Error	1.57	4	0.39			
Cor Total	1100.22	16				
R^2= 0.99				Pred R^2= 0.97		
Adj R^2= 0.99				Adeq Precision= 53.05		
Eqn. 32						

$$\text{Carbon dioxide concentration, \%} = 1196.209 - 70.6815 * (\text{Temperature, °C}) + 24.40911 * (\text{Organic Conc., g} - \text{VS}) + 2.217225 * (\text{Sludge Conc., \%}) - 0.69388 * (\text{Temperature, °C}) * (\text{Organic Conc., g} - \text{VS}) - 0.09148 * (\text{Temperature, °C}) * (\text{Sludge Conc., \%}) - 0.08435 * (\text{Organic Conc., g} - \text{VS}) * (\text{Sludge Conc., \%}) + 1.072407 * (\text{Temperature, °C})^2 + 0.558378 * (\text{Organic Conc., g} - \text{VS})^2 + 0.018081 * (\text{Sludge Conc., \%})^2 \quad \text{Eqn. 32}$$

In reference to Figure 115 and according to Figure 119, the influencing factors have an opposite effect to the behaviour they have on the methane concentration. One of the most obvious differences which can be noted from the two perturbation plots was that, the influence of the organic concentration on the carbon dioxide concentration was larger than its influence on the methane concentration.

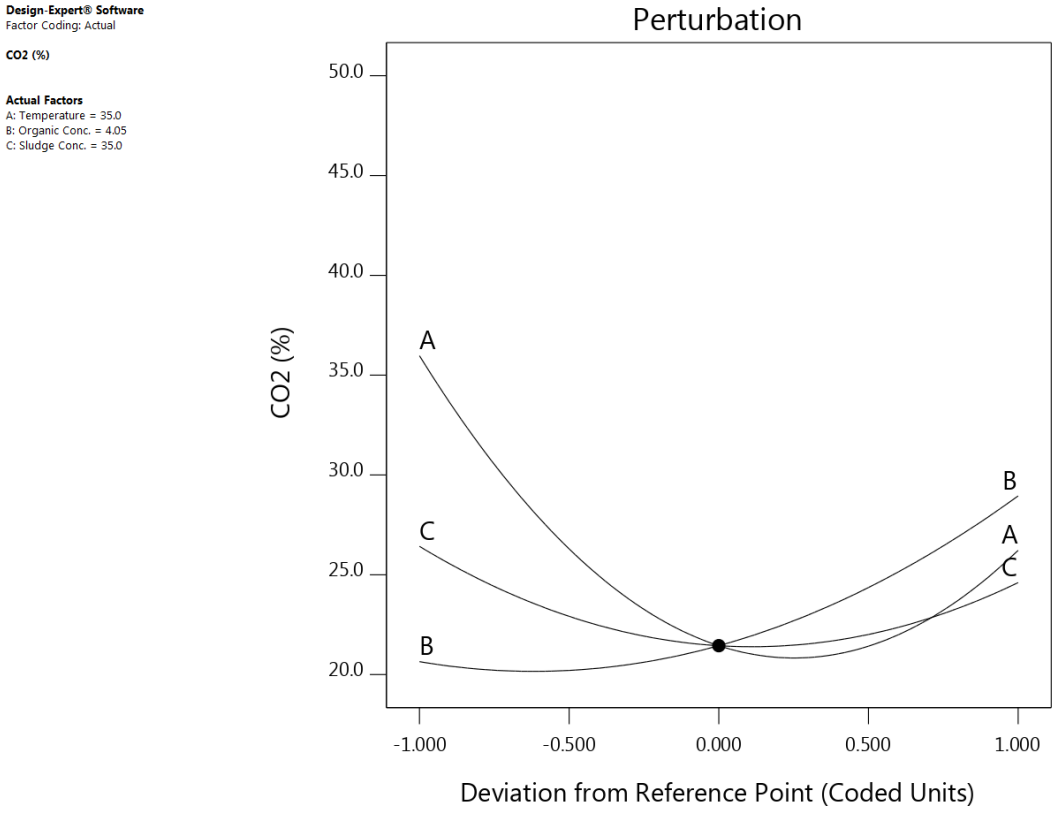


Figure 119: The Perturbation plot of the carbon dioxide concentration.

Figure 120, Figure 121 and Figure 122 show the influences of the AB, AC and BC on the carbon dioxide concentration in an interaction plots respectively. As is clear in Figure 120, the influence of the interaction of the temperature × organic concentration (AB) when the temperature was set to its highest level was insignificant. The influence of the AB increased as the temperature decreased. It can be noted that, the lowest carbon dioxide concentration was achieved, when the temperature and sludge concentration were fixed at 35°C and 35% respectively, and the organic concentration was set to its lowest level. Looking at Figure 121, it can be concluded that, the influence of the temperature and sludge concentration (AC) was more significant when the temperature was set to its highest level than when it was set to its lowest level. Moreover, when the temperature was fixed at 34 °C or a

little bit higher and the organic concentration at 4.05 g-VS, there was no influence of the AC interaction on the carbon dioxide concentration. Figure 122, confirms that the influence of the BC interaction was more significant when the organic concentration was set to its the highest level than when it was set to its lowest level. In addition, when the organic concentration adjusted to approximately 3.5 g-VS and the temperature to 35 ^{0}C, the influence of the interaction on the carbon dioxide concentration became insignificance.

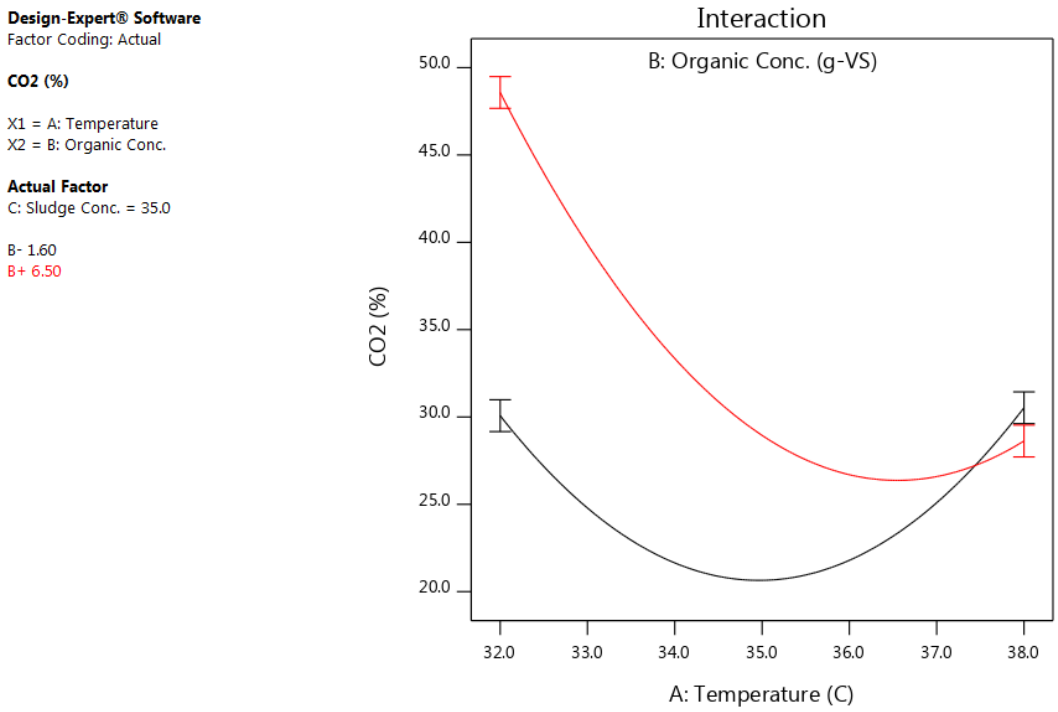


Figure 120: An interaction plot shows the influence of the temperature and organic concentration interaction on the carbon dioxide concentration.

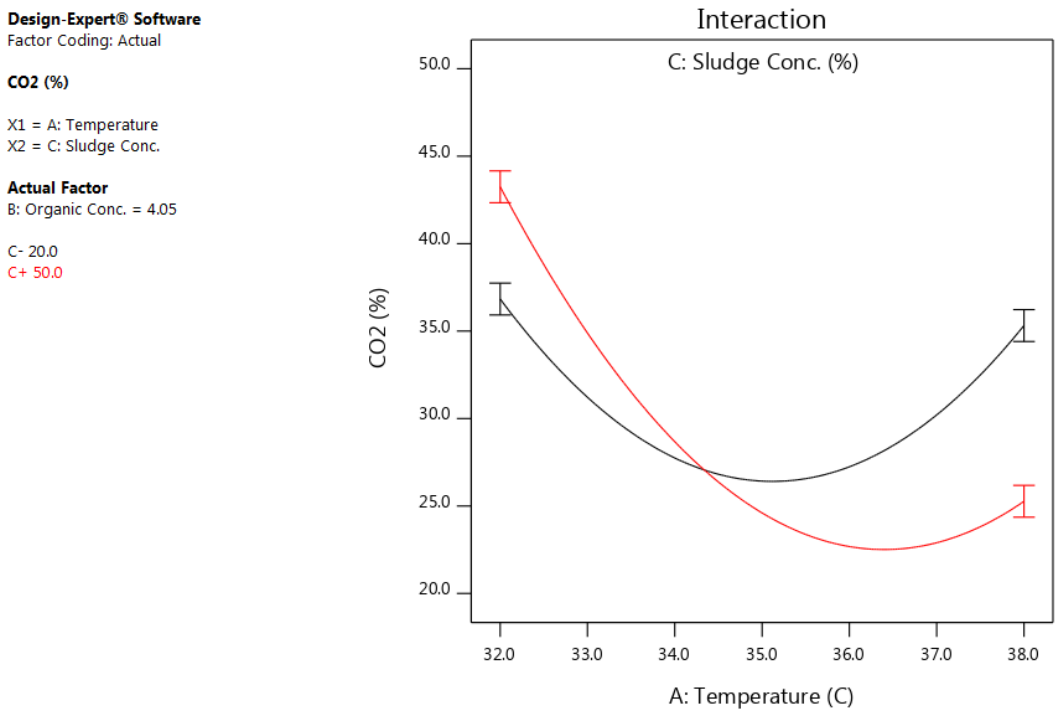


Figure 121: An interaction plot shows the influence of the temperature and sludge concentration interaction on the carbon dioxide concentration.

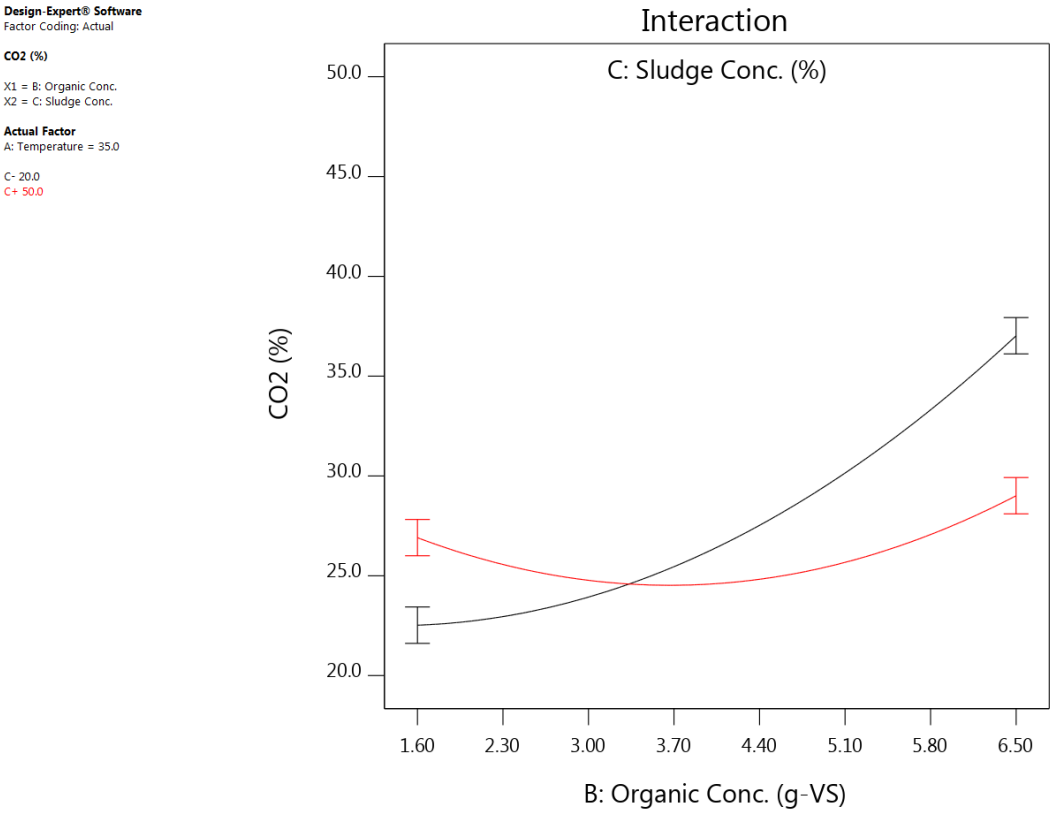


Figure 122: An interaction plot shows the influence of the organic concentration and sludge concentration interaction on the carbon dioxide concentration.

e. Methane Content In One-Gram VS

Table 34 is the ANOVA of the developed model of the methane content. It is clear that, the model was significant, its regression was good, and it can be used to navigate the design space. The analysis also showed that, the following model terms have significant influences on the methane content: A, B, C, AB, AC, A^2 and B^2 and C^2. The sludge concentration (C) had the most significant influence on the methane content. Equation 33 shows the mathematical model in terms of an actual values of this response.

Table 34: The ANOVA table for the CH_4 content of the AD of avocado residues.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	300435.9	9	33381.77	156.90	3.1E-07	significant
A-Temperature, °C	23371.64	1	23371.64	109.85	1.57E-05	
B-Organic Conc., g-VS	111488.7	1	111488.68	524.01	7.69E-08	
C-Sludge Conc., %	124635.93	1	124635.93	585.80	5.23E-08	
AB	3881.08	1	3881.08	18.24	0.003696	
AC	3449.96	1	3449.96	16.22	0.005016	
BC	1039.10	1	1039.10	4.88	0.062802	
A^2	14313.57	1	14313.57	67.28	7.77E-05	
B^2	17391.66	1	17391.66	81.74	4.14E-05	
C^2	2494.86	1	2494.86	11.73	0.01107	
Residual	1489.33	7	212.76			
Lack of Fit	1117.25	3	372.42	4.00	0.106772	not significant
Pure Error	372.08	4	93.02			
Cor Total	301925.3	16				
R^2= 0.99				Pred R^2= 0.94		
Adj R^2= 0.99				Adeq Precision= 44.59		

$$\text{Methane content}, \frac{\text{cc}}{\text{g}} - \text{VS} = -6769.27 + 431.4944 * (\text{Temperature}, °\text{C}) - 298.591 * (\text{Organic Conc.}, \text{g} - \text{VS}) - 8.7239 * (\text{Sludge Conc.}, \%) + 4.237979 * (\text{Temperature}, °\text{C}) * (\text{Organic Conc.}, \text{g} - \text{VS}) + 0.652626 * (\text{Temperature}, °\text{C}) * (\text{Sludge Conc.}, \%) + 0.438573 * (\text{Organic Conc.}, \text{g} - \text{VS}) * (\text{Sludge Conc.}, \%) - 6.47833 * (\text{Temperature}, °\text{C})^2 + 10.70706 * (\text{Organic Conc.}, \text{g} - \text{VS})^2 - 0.10819 * (\text{Sludge Conc.}, \%)^2 \quad \text{Eqn. 33}$$

Figure 123 is the perturbation plot of this response. Regardless the influences of the interactions, the sludge concentration has the most significant influence on the methane content. Following by the influences of the organic concentration and the

temperature respectively. It worth noting that, the influence of the sludge concentration on this response was oppositely to the influence of the organic concentration.

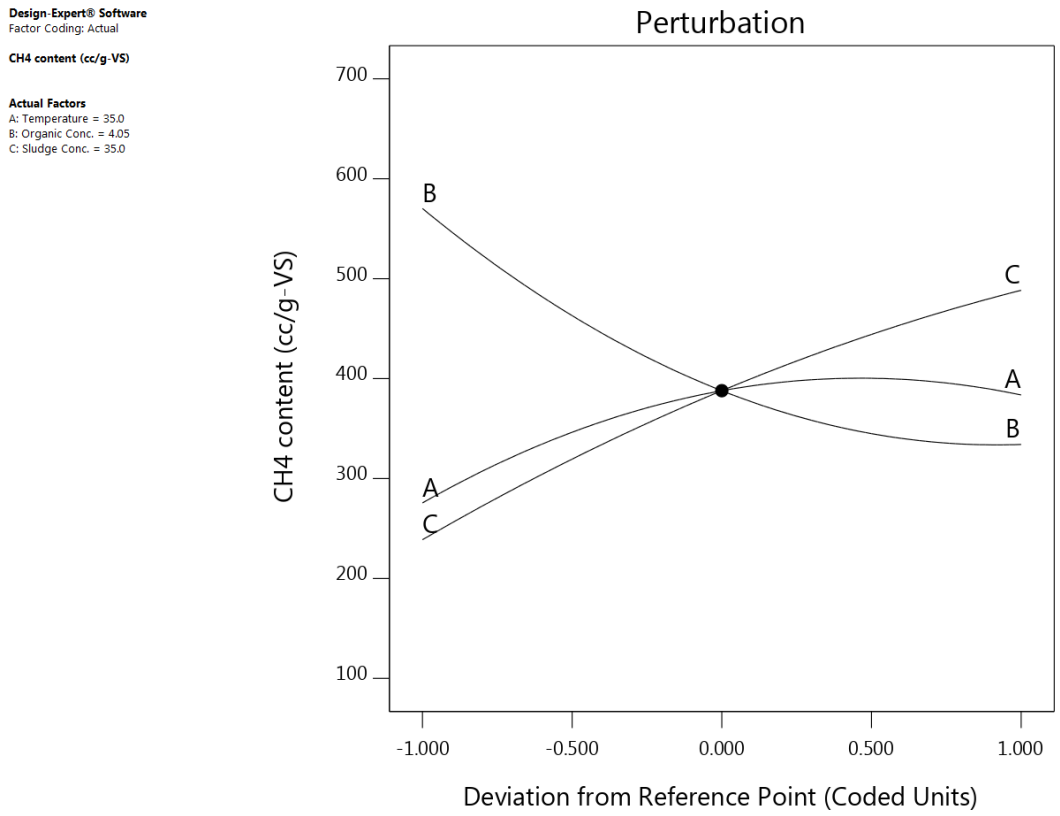


Figure 123: The Perturbation plot of the methane content.

Figure 124 and Figure 125 depict the influence of the temperature × organic concentration (AB) and the temperature × sludge concentration (AC) on this response. As is evident from the two plots, the influences of the AB was less significant when the temperature was set to its lowest level. While, the influence of the interaction of the AC was more significant when the temperature was set to its lowest level.

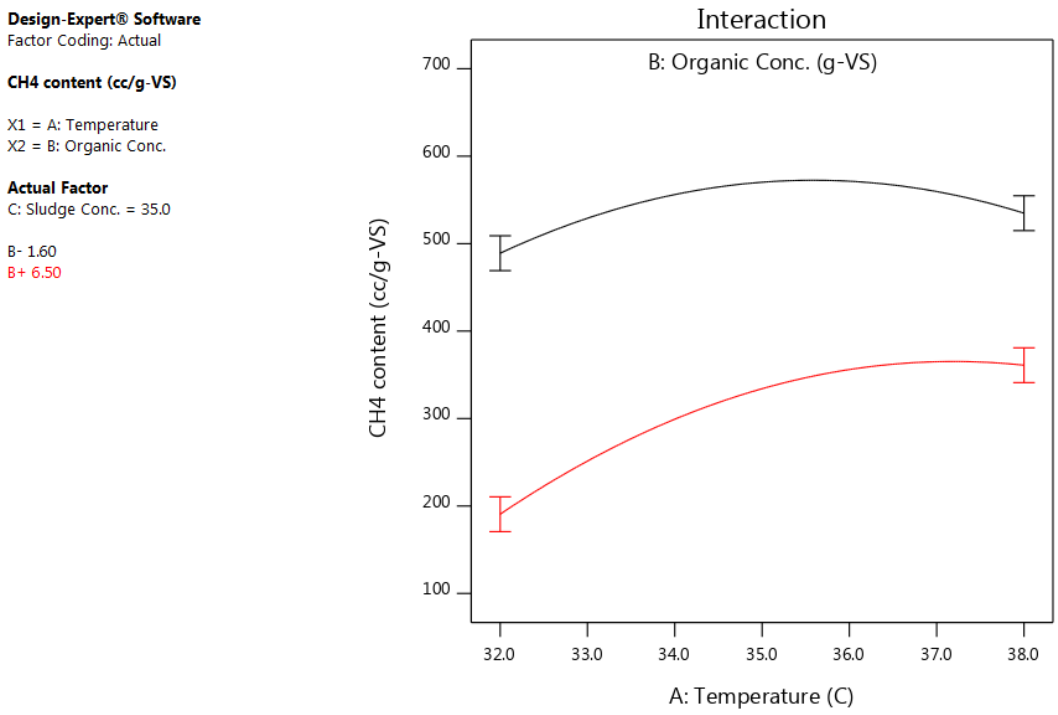


Figure 124: An interaction plot shows the influence of the temperature and organic concentration interaction on the methane content.

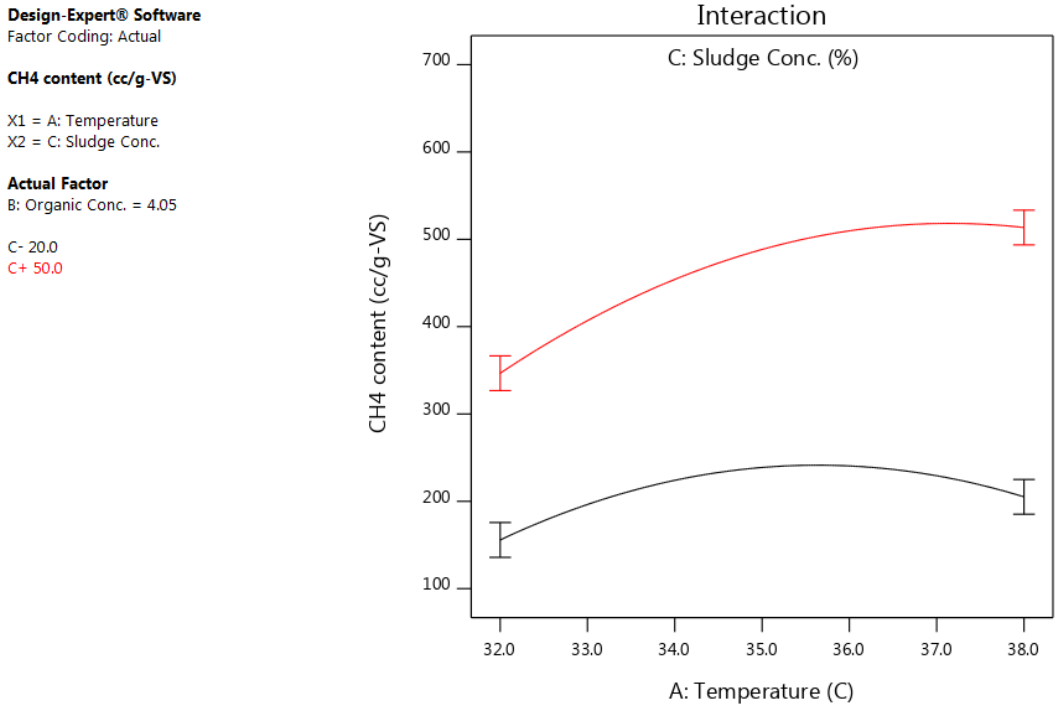


Figure 125: An interaction plot shows the influence of the temperature and sludge concentration interaction on the methane content.

2. Discussion Of The Key Findings

Persea americana "Fuerte" cultivar was used in the current study is a common variety. It is bigger than Hass, and can be distinguished from Hass by its smooth,

dark green skin and a more defined pear shape. The avocado used was processed according to the described procedure in Appendix D. The average weights of the separated peels and seeds were measured prior to AD and found equal to approximately 19% and 16% of the total fresh weight respectively. This finding is relatively close to the findings of Orhevba, B.A. and Jinadu, A.O. [301] and a 10% far from the finding of Domínguez, M.P. et al. [148] who found that, avocado seed accounts for 26% of the total fruit mass. This indicates that, the edible part of avocado constituted about two third of the total fresh weight and the remaining quantity considered residues. Additionally, the average weight of the processed avocado seed starch (ready for use) was also measured and found to account for about 20% of the dry weight of the seed. The average weight of starch obtained was quite close to the average weight of the starch extracted from the seed of "Persea americana, Miller" variety in recent studies [302, 303]. Compared to the weight of the starch extracted from the seeds in this study and the starch weights in literature, it was obvious that starch weight varies from one cultivar to another [53, 148]. According to Orhevba, B.A. and Jinadu, A.O. [301], the starch content of avocado seed depends on the cultivar used.

The biogas produced from each run was collected twice during the experiment. A drop in the biogas quantity was observed in the second collection (day 21). As noted earlier, the production of biogas increased until it is reached the peak and then decreased gradually. According to the results, the three studied influencing factors had significant influences on the quantity and quality of the biogas produced. Looking at the results of the quality of the biogas produced from the three biomass, it can be noted that, the influence of the temperature on the biogas produced from avocado residues was higher than its influences on the quality of the biogas produced from the other two biomass. Due to the higher influence of the temperature, the resulted methane concentration from the avocado residues were lower. In terms of the pH, the same trends of the pH in the AD of the other two biomass (potato residues and mango residues) were also observed in the AD of avocado residues that, the pH levels decreased by the decreases of the sludge concentration lower than the organic concentration level.

The results of the comparison between the biogas produced from the avocado residues prior to starch separation and the predicted results of the biogas after starch separation revealed that, the avocado starch has very little effect on the volume of biogas. On the other hand, its effect on the biogas quality was a little higher (see Table 29). This finding confirms the suggestion of a previous study [141], the use of the avocado seed starch in the production of biodegradable polymers for packaging of food, requires more studies to discover the potential properties of avocado seed starch, in order to utilise it in the production of other bio-products.

4.2.4 Validation Of Digestate Use In Agriculture Lands

There are variety uses of digestate, their uses depend essentially on some factors such as: the quality and the origin of the feedstock. Bio-fertiliser and soil improver are the most two common uses of digestates [36]. The EU directive 86/278/EEC (EU, 1986) governs the use of the municipal sewage sludge in agricultural lands. As per the directive, the sludge is required to be subjected to biological, chemical or heat treatment, long-term storage, or any other process to reduce the health risks that may be caused by the applications of the sludge in agriculture lands. In EU, the use of the treated sludge/digestate is based on its contents of the nutrient and metals [304].

As AD is a biological treatment process, it is important to confirm if the digestate could be applied to agriculture lands, that is to test the digestate for its bio-fertiliser potential. The resulting digestate from one of the anaerobic reactors of mango residues which was digested at 35 ^{0}C (A), 6.5 g-VS (B) and 50% (C) (i.e. run 7), produced the highest biogas volume was selected and tested. The results of the test confirmed that the content of the digestate contained the three main nutrients of fertiliser: N, P and K and found that the dry matter weight was low (see Table 35 and Appendix I). On the other hand, pH is an important factor aiding plants to absorb the nutrients, therefore the pH level was measured and found to be equal to 7.9. Based on this, the resulting digestate can be used on agriculture lands or separated into liquid and solids, and sold separately. For more assurance of the quality and validity of the digestate, the digestate would have to be tested for other elements such as; total humic and fulvic acid and heavy metals (Zn, Cu, Sn, Cd, Ni, Pb, Hg, Cr).

Table 35: The quantity of the three basic nutrients of fertiliser and dry matter weight in the digestate.

Item	quantity	Unit
Total Phosphorous	762	mg/kg
Potassium	1251	mg/kg
Total Nitrogen	3951	mg/kg
Dry matter	3.2	g/100g

Moreover, beside the low prices of digestate, there are number of factors associated with the production of the digestate which negatively affect the economic aspects of the AD, contributing to increasing the production costs of the digestate and consequently, slowing the growth of AD [36, 305]. According to P. Vilanova Plana and B. Noche [36] and the results of the digestate, the whole digestate contained low dry matter and relatively low nutrient concentrations. Due to that and because of its high content of water, the costs of the storage, transportation and application of the digestate are high. In addition to that, AD plants continuously generate digestate. These large amounts of digestate generated are not always possible to be directly applied to the agriculture lands. Therefore, sometimes storage is required for varied periods until the growth season or vegetative growth. Storing such a quantities of digestate requires AD plants to require large and appropriate facilities for storing. On the other hand, although digestate is considered a valuable fertiliser rich in important nutrients, they may also contain some harmful elements such as: antibiotic, pathogens and heavy metals. This leads to a reduction in the reliability of the digestate by some farmers and therefore limits its use. The EU directive (91/676/EEC) of 12 December 1991 controls the environmental risk regarding nitrates. For instance, ammonium-nitrogen is one of these harmful elements which may exist in the digestate and must be kept at an low level, thus regulated specify permissible amounts for such elements must be adhered to, which can be supplied to the agriculture lands [36]. Based on all these factors and others, the production cost of digestate is often higher than its value. However, the contribution of selling the digestate as a soil improver or bio-fertiliser from an economic aspect of AD, will be discussed further in this chapter.

4.2.5 Key Findings Of The AD Process

As is well-known, the objective of the AD process is not limited to the production of biogas. It also plays a crucial role in waste management. The present study discovered that, the wastes of the mango and avocado which are usually discarded, account for approximately one third of the total weight of both fruits. While, the waste of potato constitute about 12.5% of the total weight. Therefore, the processing of large amounts of such fruits and vegetables would generate behind it a large amount of waste. The accumulation of such waste would represent a major threat on the environment. Due to that and in support of what was mentioned earlier, that, food processing industries are the second largest generator of waste in the environment [50], the exploitation of the food wastes in the production of bio-products such as; biogas and bio-plastic would have significant positive impacts on the environment.

The present study revealed that the organic concentration (B, g-VS), sludge concentration (C, %) and their interaction (BC) have significant impacts on the quantity and quality of the biogas produced. The improper balancing of the concentrations of the organic concentration and sludge concentration can affect the pH level, result in low quantity and quality biogas or may even lead to a failure of the digester. This can be due to a number of reasons such as: if the growth of the bacteria was inhibited, if the metabolism of the bacteria was distorted, if the bacterial population was imbalanced or due to the accumulation of VFA [227-229]. Additionally, temperature (A, °C) had a less significant influence compared to the influences of the other two factors on the quantity and quality of the biogas, with the exception of the quality of the biogas from avocado residues. However, the microorganism's activity was sensitive to the temperature, the activity either increases/decreases due to the change in temperature. In the current study, the microorganism's activity was at the highest level when the temperature was at or close to 35 ^{0}C. This finding supports what was mentioned in Chapter 2, where most AD plants digest their substrates at temperatures between 35-37 ^{0}C [216]. Therefore, the levels of these three parameters must be carefully determined.

The finding of the comparison between the quantity and quality of the biogas produced from the three biomass before and after the separation of the starch and

mango seed coats, supports the conduction of an investigation on the incorporation of the production of bio-plastic composite sheets process with the AD process. The mango seed coats have shown a negative impact on the volume of the biogas. Therefore, they require further pre-treatment prior to applying them in the Hollander beater, or, utilising them in other applications to gain benefit of them. These conclusions greatly stimulate more explorations of the properties of mango, potato and avocado starch, mitigate the pressure on the current known sources of starch, and gaining benefit of the coats by blending them as reinforcement materials with the starch to improve the functionality of the starch.

The digestate test results proved that, the digestate contained the three main nutrients required of a fertiliser and revealed the quantity of the dry matter in the digestate. The containment of the digestate as solids (fibres) enhances its potentials to be used as it is or separated and used as a fibre or/and liquid fertiliser. The exploitation of the digestate in agricultural applications would contribute greatly in the mitigation or even limitation of the environmental issues associated with the AD process. Despite the positive impact on the environment of getting rid of digestate in selling it as a fertiliser or soil amendment, it also has negative effect on the economic feasibility of the AD due to the following challenges: (a) if the dry matter in the digestate is low and the moisture content is quite high, therefore its transportation is costly (b) if AD plants continuously generate digestate while they are producing biogas, these large amounts of the digestate cannot be sold directly. They have to be stored for varied periods until the growth seasons. The storing of such a quantity of digestate requires AD plants to build up large storages according to special standards. (c) Not all farmers are fully confident of substituting commercial fertiliser with digestate (bio-fertiliser) due to the possibility of it containing harmful elements. To fully assess the quality of the digestate, further tests need to be carried out on the digestate to measure the quantity of all contents, so as to increase its confidence amongst farmers. Due to this plus the lower prices of digestate and its production cost (including: transportation, storing, etc.) [305], the cost may exceed its value.

The primary revenues of an AD plant come from: gate fees which are set by some AD plants for farmers, to avoid landfill tax cost, the sale of the produced heat

and electricity and the sale of the digestate [305]. Because AD plants have an important role in waste management, in order to support the prosperity of AD plants, the economic profitability of AD should be enhanced. The production of more bio-products with lower production costs is one of the ways to meet these profits. Therefore, this study investigates the integration of the process of producing composite bio-plastic materials with AD process to enhance its profitability.

4.3 Bio-Plastic Sheets Production

In terms of the drying of starch, the mango starch took the longest time to become totally dried (approximately 4 days), following by avocado (approximately 3 days) and potato starch (approximately 1 day) respectively. On the other hand, after the mango and avocado starch were completely dry, they tended to be beige and light brown in colour respectively while, the potato starch remained white. The results showed using a load cell device that the average force required to fully close the mould was approximately 75 kN. Table 36 depicts the results of the four responses for each bio-plastic sheet. The analysis of each of the four responses are shown and discussed in the following sub-heading. As mentioned in the previous chapter, three bio-plastic sheets were produced separately out of the starch of each of the three biomass with no fibres, to differentiate clearly between the mechanical properties of the bio-plastic sheets produced from each of the three starch types (see Table 37). Looking at Table 37 it can be seen that, the bio-plastic sheet produced using the potato starch yielded the best mechanical properties, while, the mango bio-plastic sheet was the worst. According to Table 36 and Table 37, the comparison between the mechanical properties of the bio-plastic sheets produced from the potato starch with and without fibres indicated that mango fibres improved the three mechanical properties of the potato bio-plastic sheets. Figure 126 shows a bio-plastic sheet produced made from potato starch and mango fibres. While, Figure 127 shows the three fibre-free bio-plastic sheets produced from each of the three starch respectively.

Table 36: The results of the four responses for each bio-plastic sheet.

Run	Component 1	Component 2	Component 3	Component 4	Response 1	Response 2	Response 3	Response 4
	A: Potato	B: Mango	C: Avocado	D: Fibre	Tensile strength	Flexural stiffness	Impact strength	Density
	g	g	g	g	MPa	MPa	J/cm^2	kg/m^3
1	30.0	45.0	15.0	10.0	0.83	2.51	11.60	1770.1
2	30.0	15.0	45.0	10.0	1.36	1.94	13.39	1867.8
3	81.0	0.0	15.0	4.0	1.98	2.88	12.74	1773.6
4	62.0	0.0	30.0	8.0	1.77	3.13	16.33	1837.6
5	90.0	0.0	0.0	10.0	2.25	3.45	18.87	1774.4
6	24.0	33.8	33.8	8.5	0.78	2.06	12.69	1973.7
7	81.0	15.0	0.0	4.0	1.77	2.73	12.84	1797.4
8	62.0	30.0	0.0	8.0	1.42	2.10	12.12	1728.5
9	4.0	45.0	45.0	6.0	0.54	0.71	12.32	1841.9
10	51.0	22.5	22.5	4.0	0.95	2.59	12.48	1772.2
11	51.0	22.5	22.5	4.0	0.93	2.34	12.22	1712.3
12	46.5	33.8	11.3	8.5	1.28	3.56	12.07	1790.2
13	46.5	11.3	33.8	8.5	1.33	2.70	11.76	1831.4
14	62.0	30.0	0.0	8.0	1.50	1.74	11.45	1769.2
15	51.0	45.0	0.0	4.0	1.01	2.07	12.12	1651.1
16	94.0	0.0	0.0	6.0	2.36	1.73	18.44	1650.1
17	4.0	45.0	45.0	6.0	0.62	0.71	12.69	1871.7
18	51.0	22.5	22.5	4.0	0.97	2.94	12.84	1798.1
19	51.0	0.0	45.0	4.0	1.92	2.86	12.41	1917.8
20	62.0	0.0	30.0	8.0	1.60	3.81	12.31	1802.2

Table 37: The results of the mechanical properties of the fibres-free bio-plastic sheets produced from the three starch separately.

Starch type	Tensile strength	Flexural stiffness	Impact strength
	MPa	MPa	J/cm^2
Potato	2.22	1.85	12.63
Mango	0.52	1.35	12.12
Avocado	1.11	1.50	12.48

Figure 126: A bio-plastic sheet produced, made from potato and fibres.

Figure 127: The fibre-free bio-plastic sheet produced from (a) potato starch, (b) mango starch, and (c) avocado starch.

4.3.1 Analysis Of The Responses

1. Tensile Strength

The tensile strength tests were carried out for each bio-plastic sheet in triplicate and the averages were calculated. The tests were performed using a Zwick's 5 kN universal electro-mechanical testing machine model Z5. The results of the tensile strength tests revealed that the highest tensile strength obtained was 2.36 MPa for the bio-plastic sheet made from 94 g potato starch and 6 g mango fibres. In addition, the lowest tensile strength was 0.54 MPa which resulted from the bio-plastic sheet made of 4 g potato starch, 45g mango starch, 45g avocado starch and 6 g mango fibres. Figure 128 shows the failure mode of the tensile strength test specimens.

Figure 128: The failure mode of the tensile strength test specimens.

The ANOVA analysis in Table 38 shows that, the developed model and the following model terms: potato (A), avocado (C), interaction of potato and mango (AB), interaction of potato and avocado (AC), interaction of mango and avocado (BC) and interaction of mango starch and mango fibres (BD) were significant. The lack of fit was not significant and the model adequately fit the data. Moreover, the R^2, adjusted R^2, and predicted R^2 were all close to 1 thus, the regression of the model was good. The "Pred R^2" of 0.94 was in reasonable agreement with the "Adj R^2" of 0.97. The “Adeq Precision” was greater than 4. Therefore, the model adequately fit the data and was used to navigate the design space. According to Table 38, the potato × avocado (AC term) has the highest influence on the tensile strength. Equation 34 shows the mathematical model of this response in terms of an actual values.

Table 38: The ANOVA table of the tensile strength response.

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	5.28	7	0.7543	97.34	< 0.0001	significant
Linear Mixture	4.75	3	1.58	204.41	< 0.0001	
AB	0.0766	1	0.0766	9.88	0.0085	
AC	0.3387	1	0.3387	43.71	< 0.0001	
BC	0.3123	1	0.3123	40.30	< 0.0001	
BD	0.0724	1	0.0724	9.34	0.0100	
Residual	0.0930	12	0.0077			
Lack of Fit	0.0716	7	0.0102	2.39	0.1773	not significant
Pure Error	0.0214	5	0.0043			
Cor Total	5.37	19				
R^2= 0.98				Pred R^2 = 0.95		
Adj R^2 = 0.97				Adeq Precision= 32.32		

$$\text{Tensile strength} = +0.025733 * (\text{Potato starch}) + 0.002033 * (\text{Mango starch}) + 0.044763 * (\text{Avocado starch}) - 0.009176 * (\text{Mango Fibre}) - 0.000290 * (\text{Potato starch}) * (\text{Mango starch}) - 0.000621 * (\text{Potato starch}) * (\text{Avocado starch}) - 0.000939 * (\text{Mango starch}) * (\text{Avocado starch}) + 0.001764 * (\text{Mango starch}) * (\text{Mango Fibre}) \quad \text{Eqn. 34}$$

Figure 129 and Figure 130 are the residual normality plot and the predicted values versus the actual values plot respectively. Figure 129 indicates that the residuals are normally distributed. Figure 130 illustrates that the distribution of most of the points was on the diagonal line or close to it therefore, the model predicted the results very well. This means, there was a good correlation between the model's predicted results and the actual results.

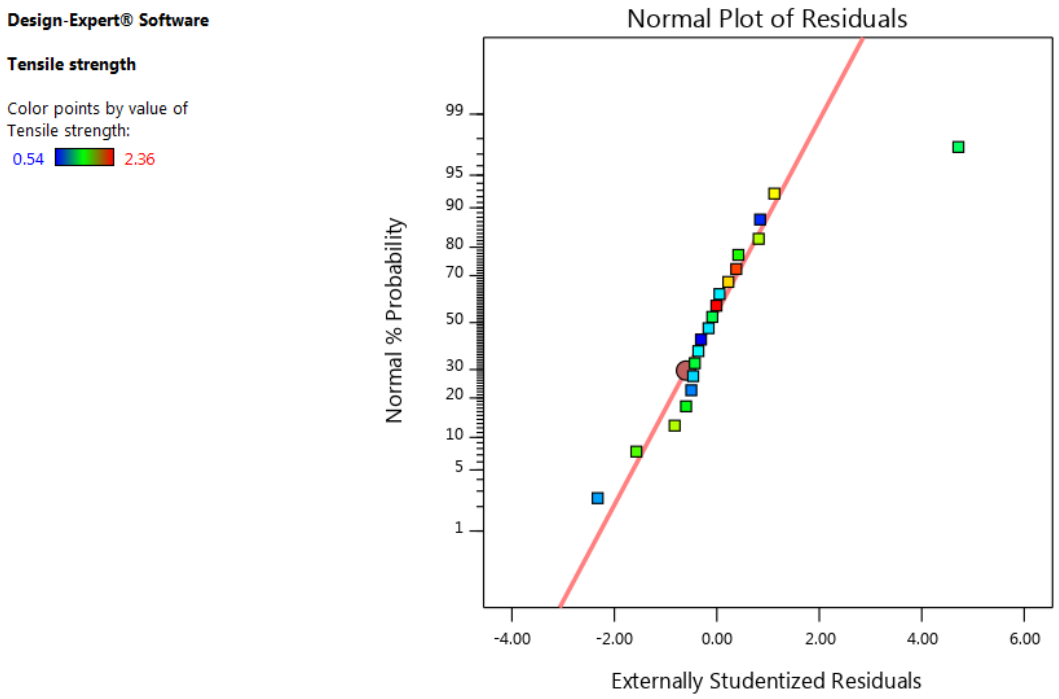


Figure 129: The normal plot of residuals of the results of the tensile strength response.

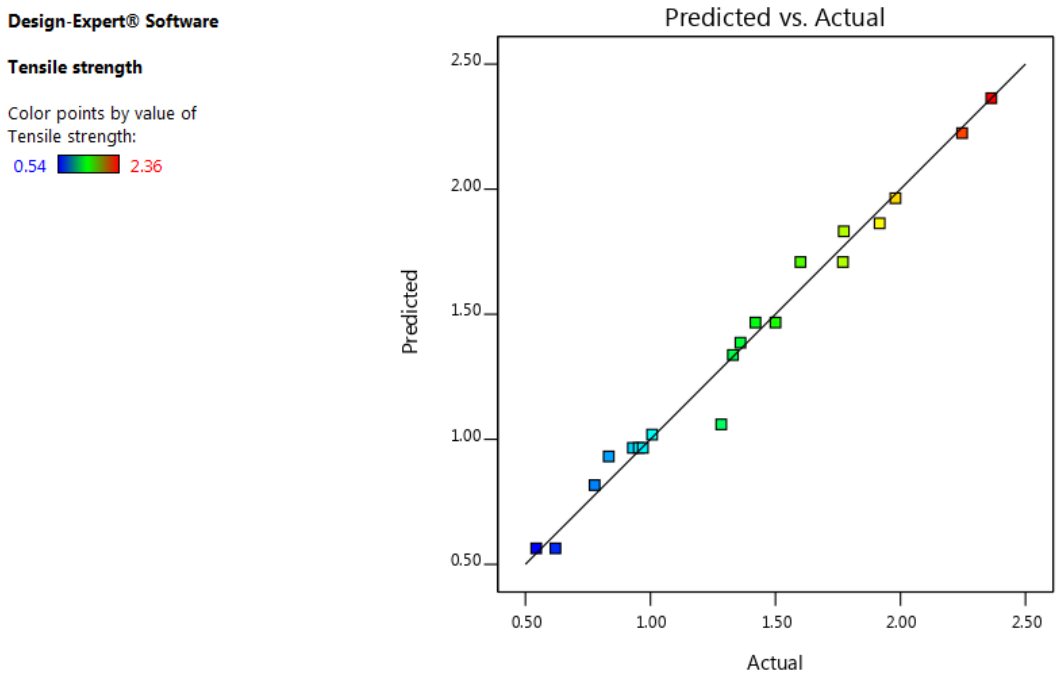


Figure 130: The scatter plot of the predicted values versus the actual values of the tensile strength response.

The Trace (Piepel) plot (Figure 131) shows the behaviour of each component with respect to tensile strength. It is clear, the tensile strength sharply increases with the increasing of the potato starch quantity (A) after the deviation point. The plot also showed that, the tensile strength decreases as the mango starch quantity (B)

increases, while, it increases gradually with any increase in the avocado starch amount (C) after the deviation point. A slight increasing pattern can also be observed in the tensile strength when the mango fibre amount (D) increases. This slight increase was because the studied range of the mango fibre quantity (4 to 10 g) was relatively small. In order to predicting the tensile strength outside of the studied range of the mango fibre quantity, Equation 34 can be applied.

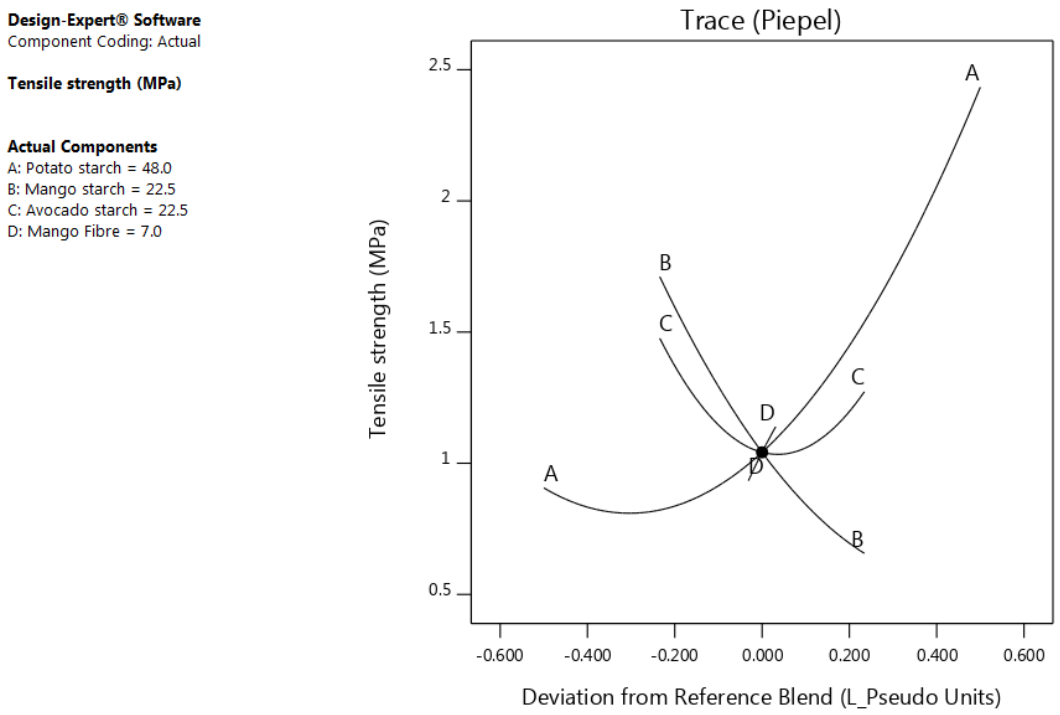


Figure 131: The Trace plot of the tensile strength response.

The two component mix plot illustrates the influence of the blending of two components on a response. Figure 132 shows the impact of the interaction of potato and mango starch quantity (AB) on the tensile strength when the avocado starch quantity and mango fibre quantity were fixed at 22.5 and 7 g respectively. The figure reveals that, the interaction is significant when the potato starch quantity is with a range of 25.5 to 70.5 g and mango starch with a range of 0 to 45 g. It is evident that, a decrease of the mango starch quantity (B) was accompanied by an increase in the tensile strength, while, an increase in the potato starch amount (A) resulted in an increase in the tensile strength. Figure 133 shows the influence of the interaction of potato and avocado starch quantity (AC) on the tensile strength. Comparing Figure 132 and Figure 133, it can be noted that, the changing in the tensile strength was less when the avocado starch amount (C) increased/ decreased.

In addition, the two component mix plot in Figure 134 shows that, at (A) = 48 g and (D) = 7 g, the highest tensile strength can be reached at about 1.84 MPa. According to Figure 134, this can be obtained by setting the mango starch amount (B) to 0 g and the avocado starch amount (C) to 45 g. Moreover, Figure 135 shows the influence of the blending of quantity of mango starch and fibre (BD) on the tensile strength when the potato and avocado starch quantities are fixed at their mid points. It is clear, the tensile strength is inversely proportion with mango starch quantity (B) and directly proportion with the mango fibre quantity (D).

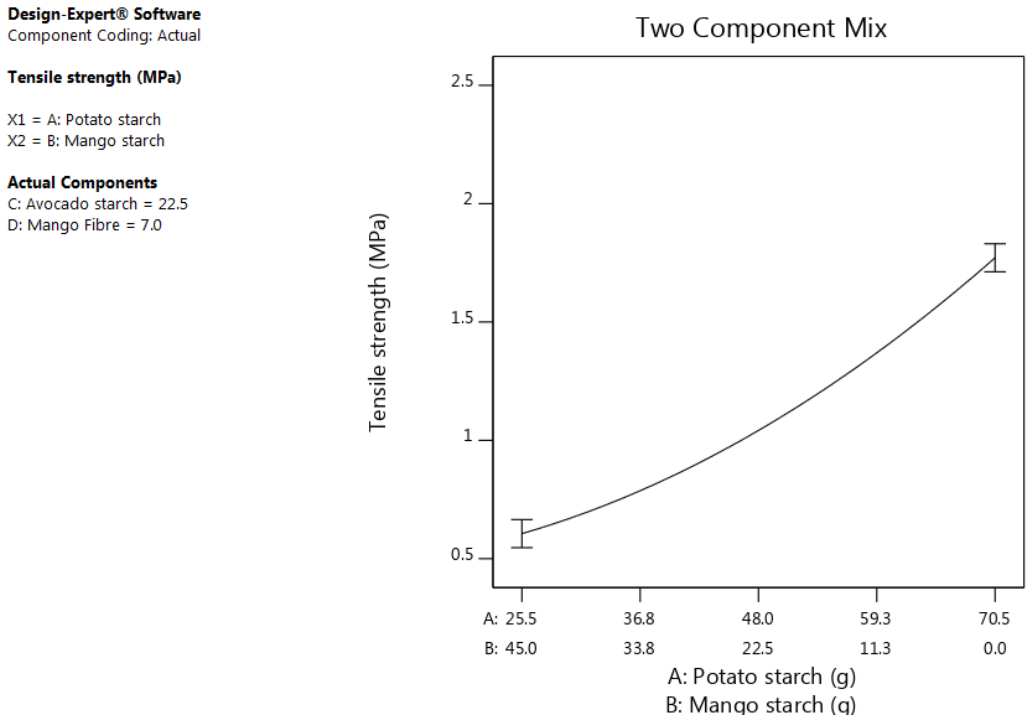


Figure 132: Two component mix plot shows the influence of the potato starch and mango starch (AB) on the tensile strength.

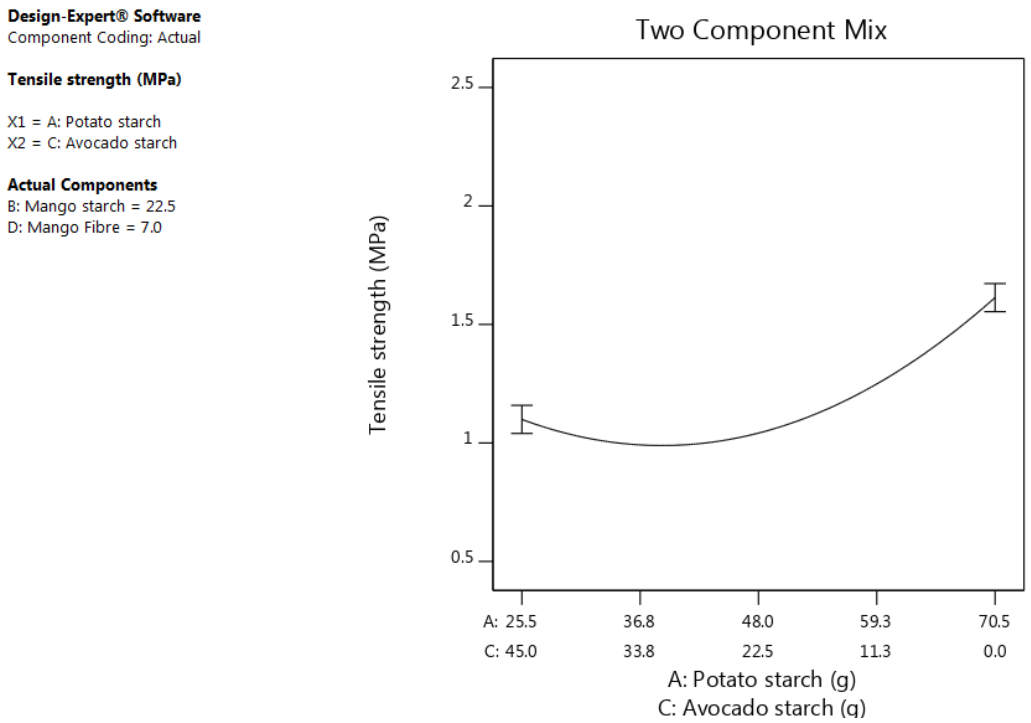


Figure 133: Two component mix plot shows the influence of the potato starch and avocado starch on the tensile strength.

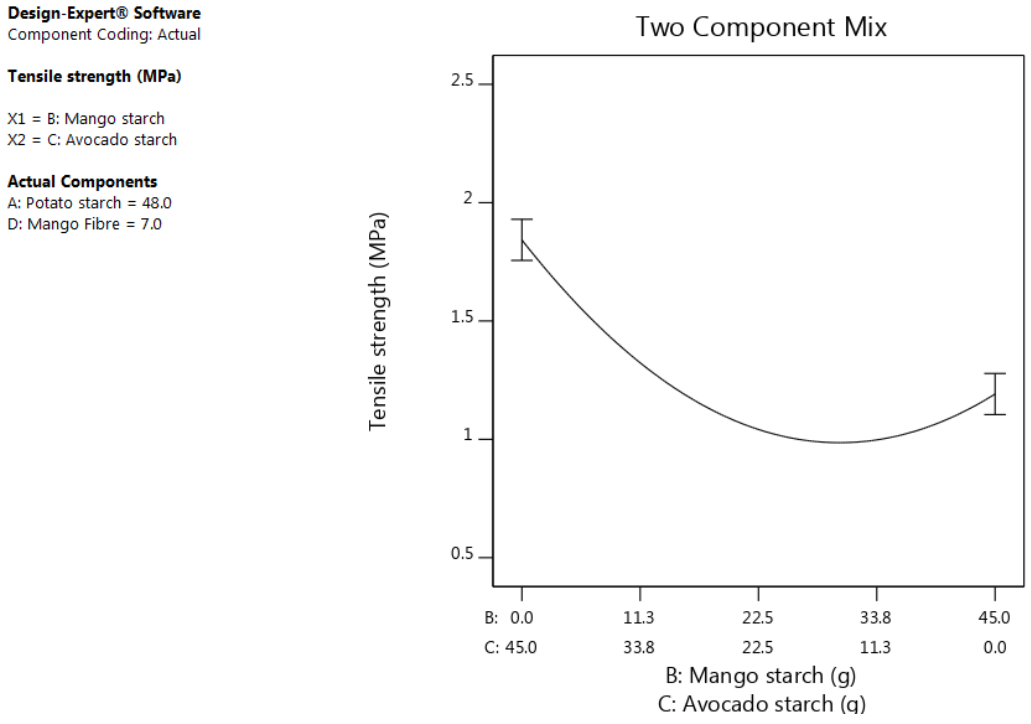


Figure 134: Two component mix plot shows the influence of the mango starch and avocado starch on the tensile strength.

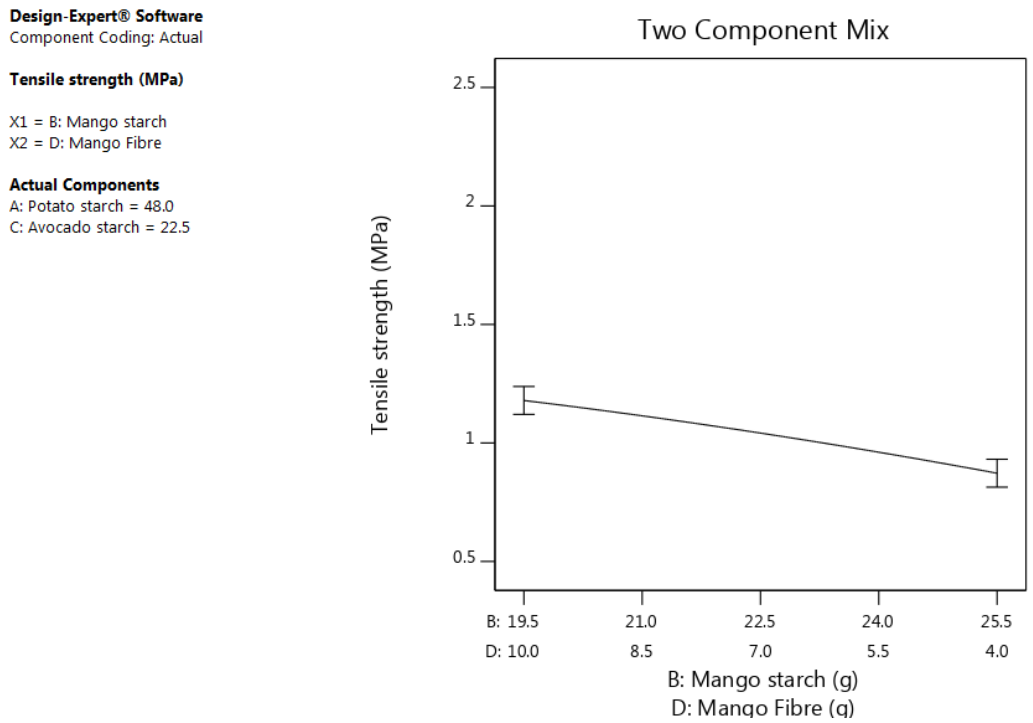


Figure 135: Two component mix plot shows the influence of the mango starch and mango fibre on the tensile strength.

2. ***Flexural Stiffness***

The bending tests were carried out on all samples using the same machine used for carrying out the tensile strength test. Table 36 shows that, the highest bending stiffness obtained was by blending 62 g of potato starch, 30 g avocado starch and 8 g mango fibres. The ANOVA analysis in Table 39 confirmed that the developed model is significant and following model terms have significant influences on the bending stiffness: A, B, D, AC. The analysis also showed that, the R^2, adjusted R^2, and predicted-R^2 were a little bit further from 1 which probably occurred due to noise in data. Despite that, the analysis also revealed that the Pred. R^2 was in reasonable agreement with the Adj. R^2 (< 0.2). The model adequately fit the data, the regression of the model was good enough and the model was able to navigate the design space. Equation 35 shows the mathematical model in terms of an actual values of this response.

Table 39: The ANOVA table of the flexural stiffness response.

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	9.56	4	2.39	10.26	0.0003	significant
Linear Mixture	5.36	3	1.79	7.67	0.0025	
AC	4.20	1	4.20	18.04	0.0007	
Residual	3.49	15	0.2329			
Lack of Fit	3.01	10	0.3013	3.13	0.1098	not significant
Pure Error	0.4811	5	0.0962			
Cor Total	13.05	19				
R^2= 0.73				Pred R^2 = 0.49		
Adj R^2 = 0.66				Adeq Precision= 11.41		

$$\text{Flexural stiffness} = +0.018933 * (\text{Potato starch}) + 0.018190 * (\text{Mango starch}) - 0.023865 * (\text{Avocado starch}) + 0.100267 * (\text{Mango fibre}) + 0.001141 * (\text{Potato starch}) * (\text{Avocado starch}) \quad \text{Eqn. 35}$$

Figure 136 shows the normal residual plot of the response. It confirms that the residuals were normally distributed. The predicted values versus the actual values plot (Figure 137) shows the results and was used to check for constant errors. Despite the existence of some outliers, there was a general correlation between the predicted and the actual results of the model.

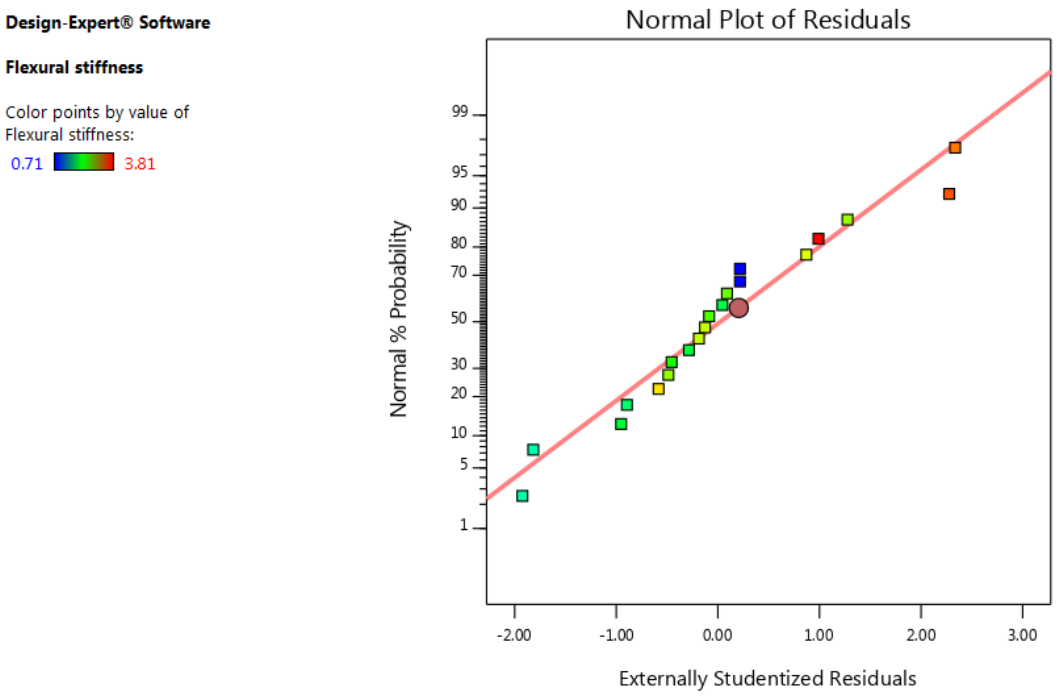


Figure 136: The normal plot of residuals of the results of the flexural stiffness response.

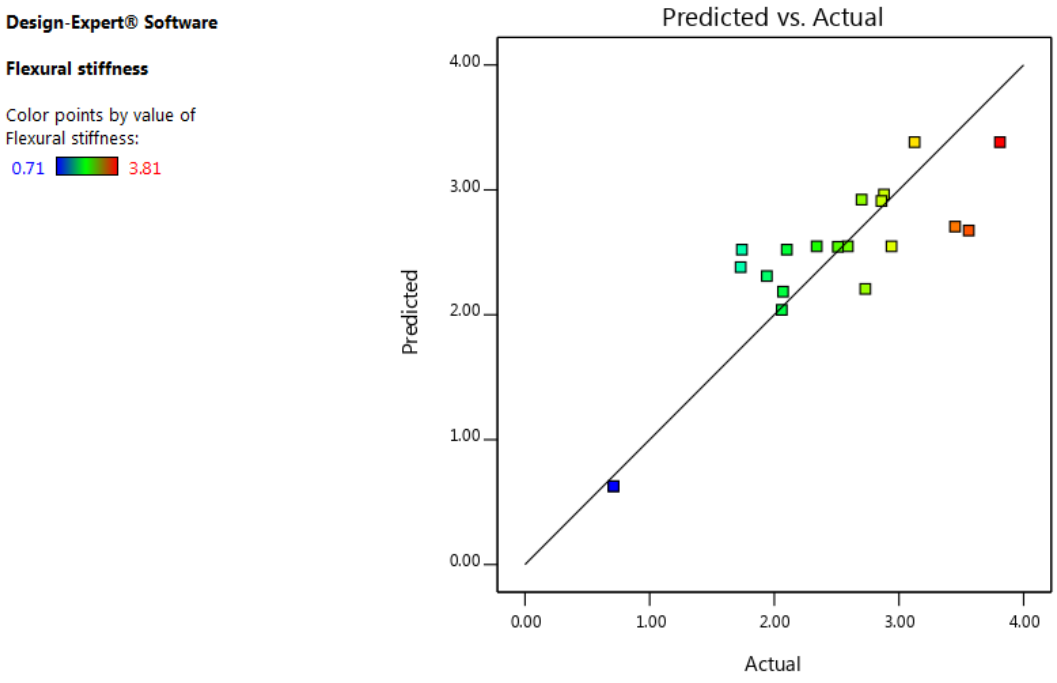


Figure 137: The scatter plot of the predicted values versus the actual values of the flexural stiffness response.

The Trace (piepel) plot in Figure 138 illustrates that, the flexural stiffness increases as the potato starch quantity (A) increases until the starch quantity reaches approximately 62 g and then decreases with further increase of the potato starch quantity (A). Mango starch and fibres quantities (B) and (D) showed quite similar

behaviours to their behaviours during tensile strength. From this figure, it can be also noted that, for 22.5 g or greater amounts of avocado starch (C), the flexural stiffness decreases and vice versa. On the other hand, Figure 139 shows the influence of the (AC) on the flexural stiffness. It is evident from the plot that, the influence of the (AC) is significant when the potato starch quantity (A) ranges from 22.5 to 70.5 g. The plot also showed that, when the mango starch and fibres quantities (B) and (D) are set to their centre points, the lowest flexural stiffness is achieved by mixing of 25.5 g potato starch (A) with 45 g avocado starch (C).

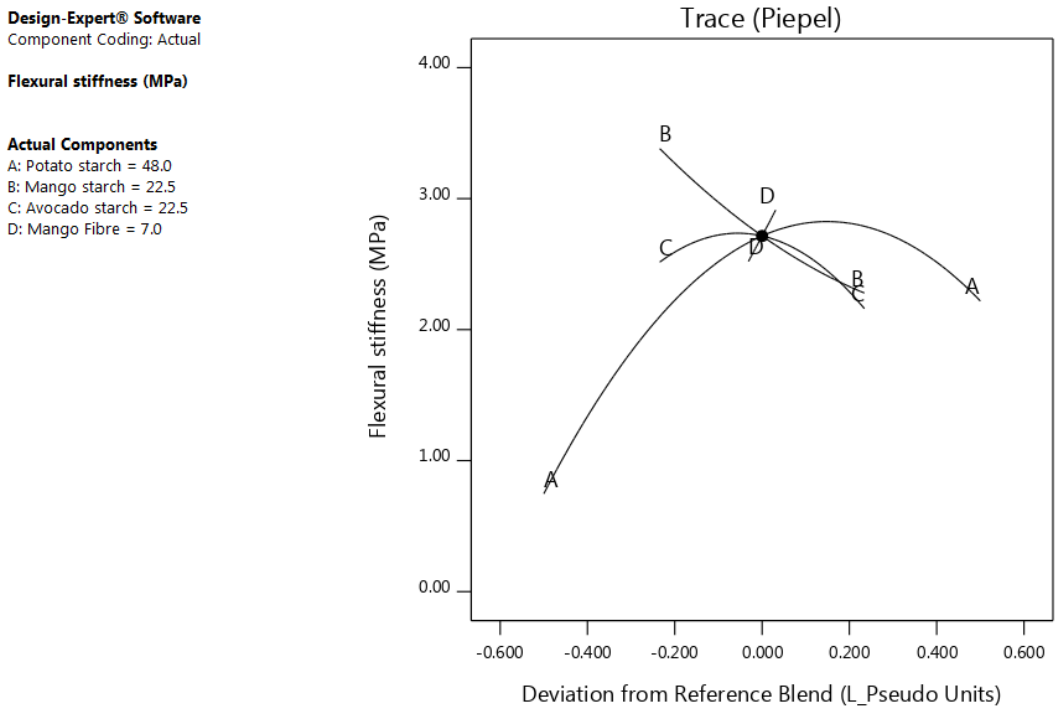


Figure 138: The Trace plot of the flexural stiffness response.

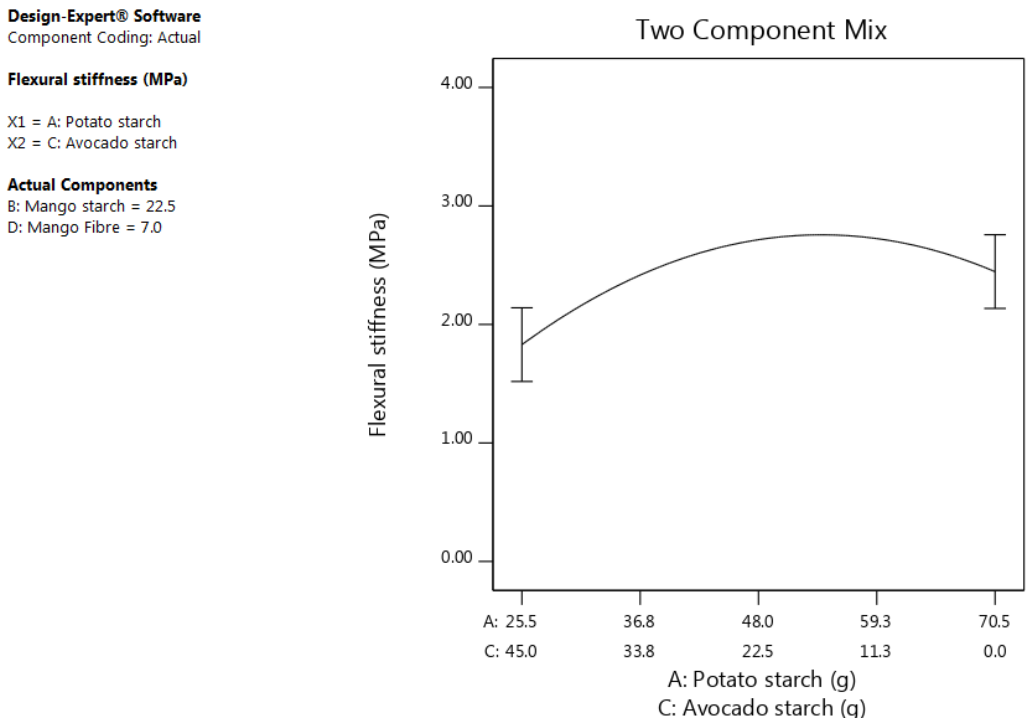


Figure 139: Two component mix plot shows the influence of the potato starch and avocado starch on the flexural stiffness.

3. Impact Loading Strength

The results of the impact loading strength tests showed that, the highest impact strength was obtained by mixing of 90 g of potato starch (A) with 10 g of mango fibres (D). Table 40 shows the ANOVA analysis of the impact loading strength response, which confirmed that the model was significant. As is shown in the table, the model terms A, B, C, D, AB, BD are significant model terms. The analysis revealed that, the R^2, adjusted R^2, and predicted R^2 were all not very close to 1 but, the regression of the model was adequate. This can be attributed to that, testing machine used displays the data using an analog scale. As a result the data were relatively close to each other, thus noise in data occurred. Based on the analysis, the "Pred R-Squared" was in reasonable agreement with the "Adj R-Squared" (< 0.2) and the "Adeq Precision" was greater than 4. Therefore, it can be said that the model was adequately fit the data and able to be used to navigate the design space. Moreover, the model term (D) had the most significant influence on the impact loading strength response. Equation 36 shows the mathematical model in terms of an actual values of this response as computed by the software. Figure 140 shows three test specimens after testing their impact loading strength.

Table 40: The ANOVA table of the impact loading strength response.

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	64.07	5	12.81	8.39	0.0008	significant
Linear Mixture	34.57	3	11.52	7.54	0.0031	
AB	21.31	1	21.31	13.95	0.0022	
BD	7.11	1	7.11	4.65	0.0489	
Residual	21.38	14	1.53			
Lack of Fit	12.85	9	1.43	0.8369	0.6163	not significant
Pure Error	8.53	5	1.71			
Cor Total	85.45	19				
R^2= 0.75				Pred R^2 = 0.52		
Adj R^2 = 0.66				Adeq Precision= 10.40		

$$\text{Impact strength} = +0.139816 * (\text{Potato starch}) + 0.256002 * (\text{Mango starch}) + 0.049578 * (\text{Avocado starch}) + 0.589374 * (\text{Mango fibre}) - 0.002572 * (\text{Potato starch}) * (\text{Mango starch}) - 0.016543 * (\text{Mango starch}) * (\text{Mango Fibre}) \quad \text{Eqn. 36}$$

Figure 140: Test specimens after testing their impact strength.

Figure 141 illustrates the distribution of the residuals. The distribution of the points were almost linear. Thus, it is possible to say that the residuals were normally distributed. This means the residuals are symmetrical about the mean. As shown in

the predicted values versus the actual values plot (Figure 142), despite the noise of data occurred, there was a general correlation as the Pred R^2 - Adj R^2 was < 0.2.

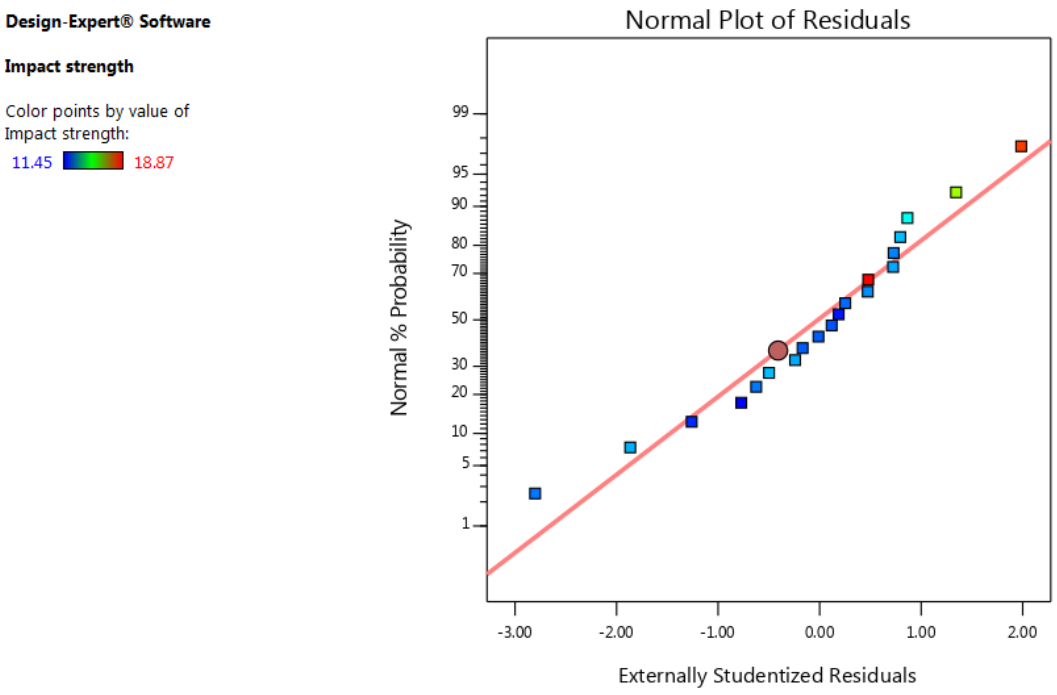


Figure 141: The normal plot of residuals of the results of the impact loading strength response.

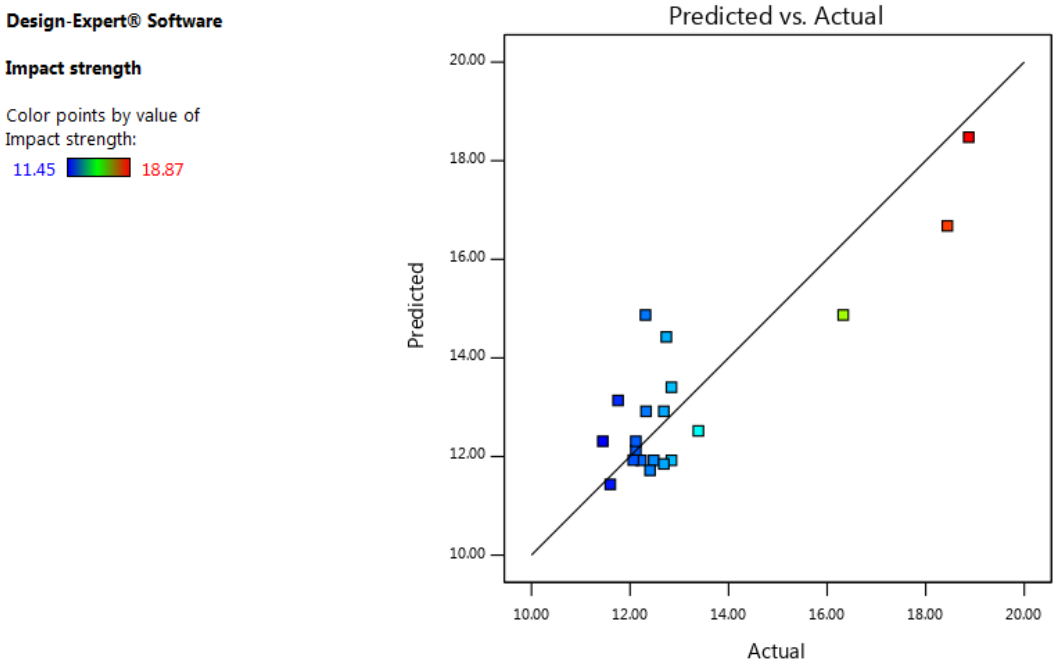


Figure 142: The scatter plot of the predicted values versus the actual values of the impact loading strength response.

According to Figure 143, the influence of the mango starch and fibre quantities (B) and (D) were similar to their influences on the tensile and flexural strengths. In

addition, the behaviour of the potato starch quantity (A) was quite similar also to its behaviour during the tensile strength response. Avocado starch quantity (C), however has an inverse relationship on impact strength.

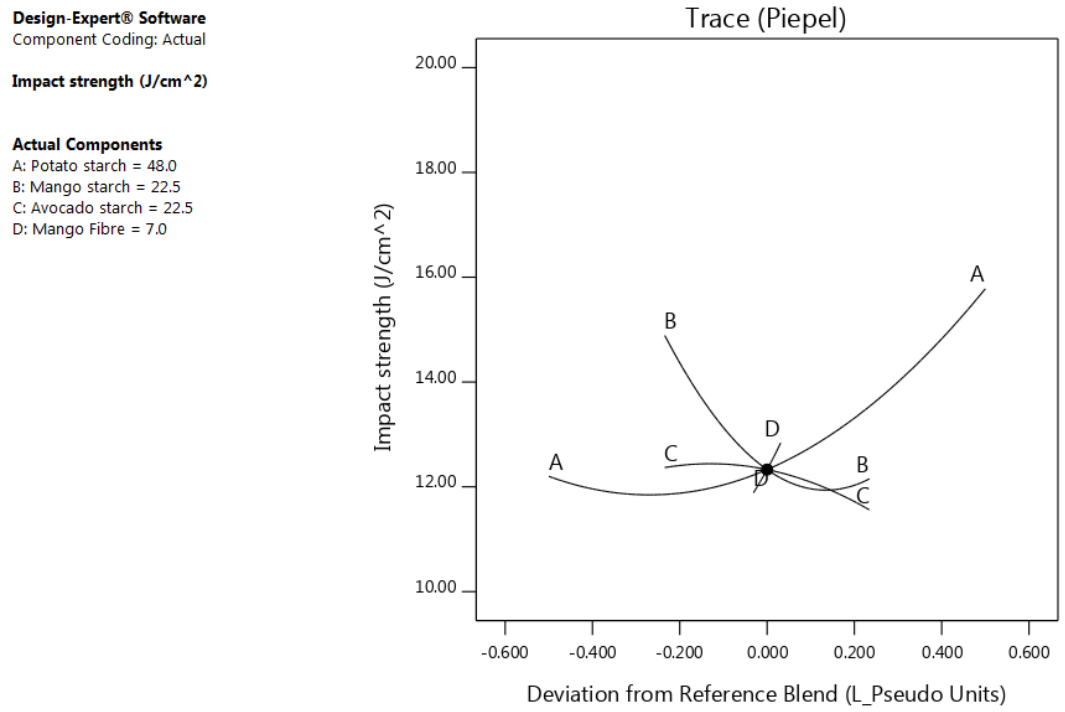


Figure 143: The Trace plot of the impact loading strength response.

Figure 144 illustrates the influence of the model term AB on the impact strength. It is evident from the figure, that the highest impact strength can be achieved when the avocado starch (C) and mango fibres (D) quantities are set to their centre points by blending them with 70.5 g of potato starch. Figure 145 shows that, when the potato (A) and avocado (C) starch quantities are fixed to their centre points and the mango starch quantity (B) is set between 19.5 to 25.5 g, any increase in the mango fibre is accompanied by an increase in the impact strength.

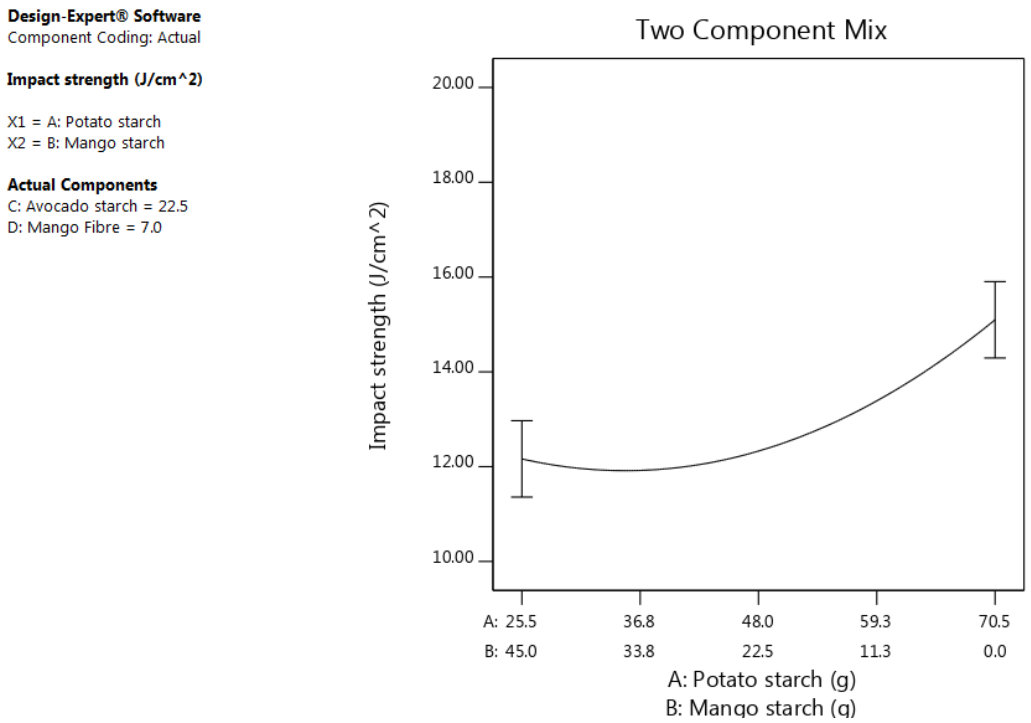


Figure 144: Two component mix plot shows the influence of the potato starch and mango starch on the impact strength.

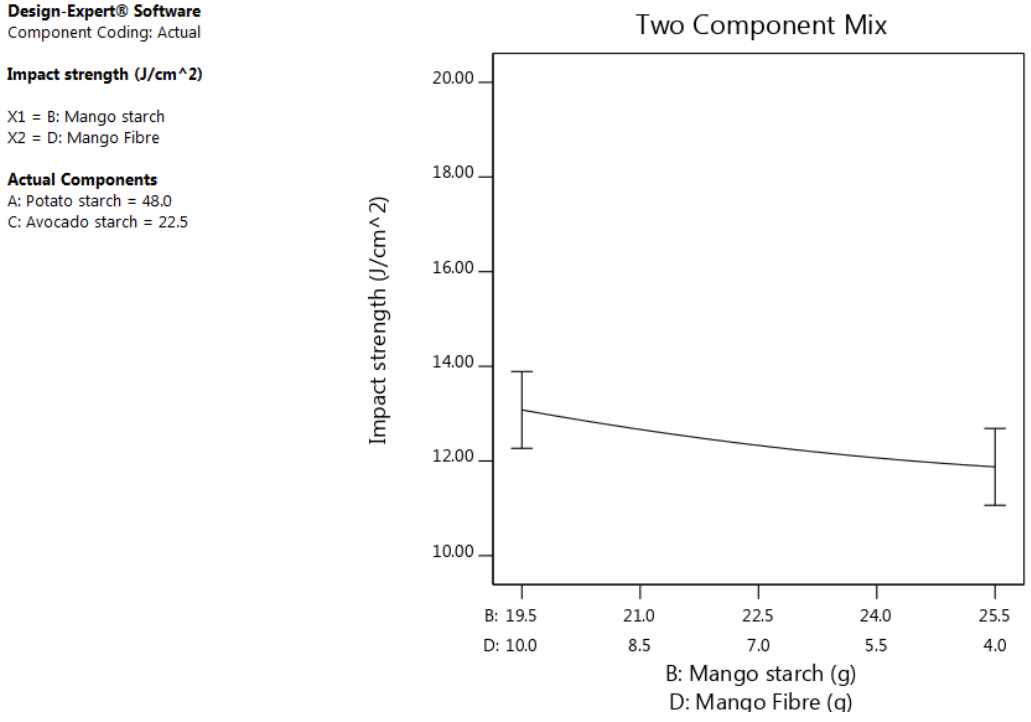


Figure 145: Two component mix plot shows the influence of the mango starch and mango fibre on the impact strength.

4. Density

Density is an important physical property of any polymer. In the present study, knowing the density greatly helps in calculating the cost effectiveness of the

production of the bio-plastic sheets. As mentioned in the previous chapter, the density tests were measured for all specimens using a Pycnometer device. The device measures the density of each specimen repeatedly and then calculates the average to minimise the error. The ANOVA (Table 37) checked the adequacy of the developed model and confirmed that, the model was significant and the lack of fit was insignificant. The analysis also showed that the model terms A, B, C and D had significant influences on density. Moreover, the ANOVA revealed that the predicted R^2 of 0.40 is in reasonable agreement with the Adjusted R^2 of 0.54 (< 0.2). However, the R^2, adjusted R^2, and predicted R^2 were far than 1 but the regression of the model was reasonably good. This may occur due to the un-evenly distribution of starch and fibre over the entire sheet, as discussed in section 4.3.2. Additionally, there was no significant influences of any interaction on the density (see Equation 37).

Table 41: The ANOVA table of the impact loading strength response.

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	72783.28	3	24261.09	8.36	0.0014	Significant
Linear Mixture	72783.28	3	24261.09	8.36	0.0014	
Residual	46446.15	16	2902.88			
Lack of Fit	40673.82	11	3697.62	3.20	0.1045	not significant
Pure Error	5772.33	5	1154.47			
Cor Total	1.192E+05	19				
R^2= 0.61				Pred R^2 = 0.40		
Adj R^2 = 0.54				Adeq Precision= 8.48		

$$\text{Density} = +16.79867 * (\text{Potato starch}) + 16.61425 * (\text{Mango starch}) + +20.23680 * (\text{Avocado starch}) + +24.13651 * (\text{Mango fibre}) \quad \text{Eqn. 37}$$

The normal plot of residuals (Figure 146) shows that almost all points were located on the line or close to it. This is indicating that the residuals are normally distributed. Furthermore, Figure 147 illustrates the predicted values versus the actual values plot. According to the plot, that there was a reasonable correlation between the predicted and the actual results despite the existence of some outliers which perhaps occurred due to the reason previously clarified.

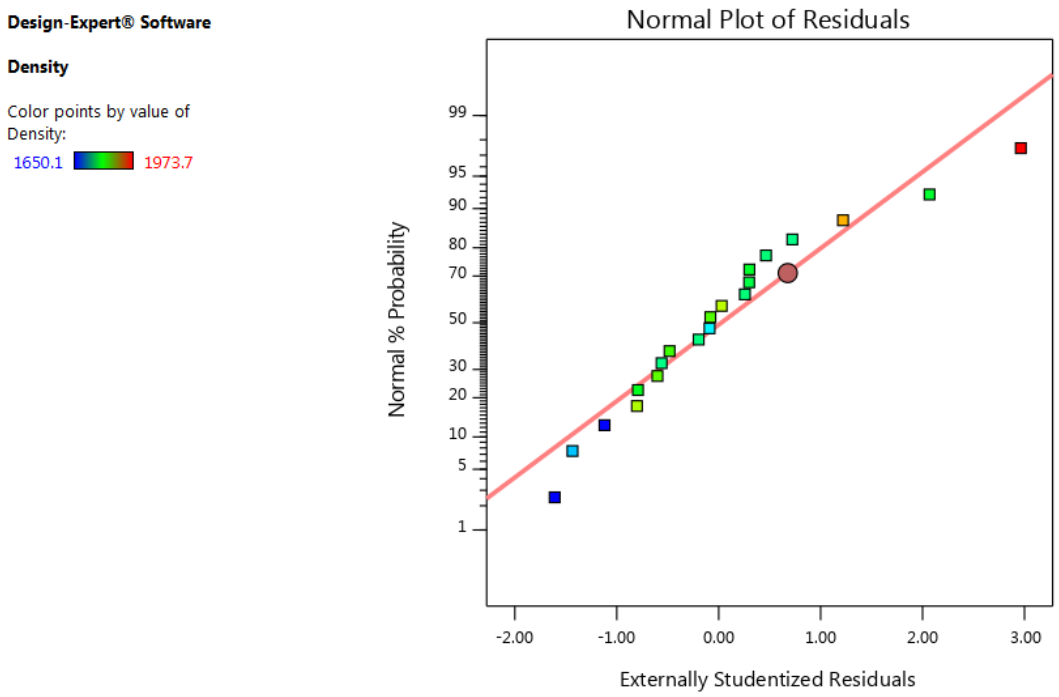


Figure 146: The normal plot of residuals of the results of the density response.

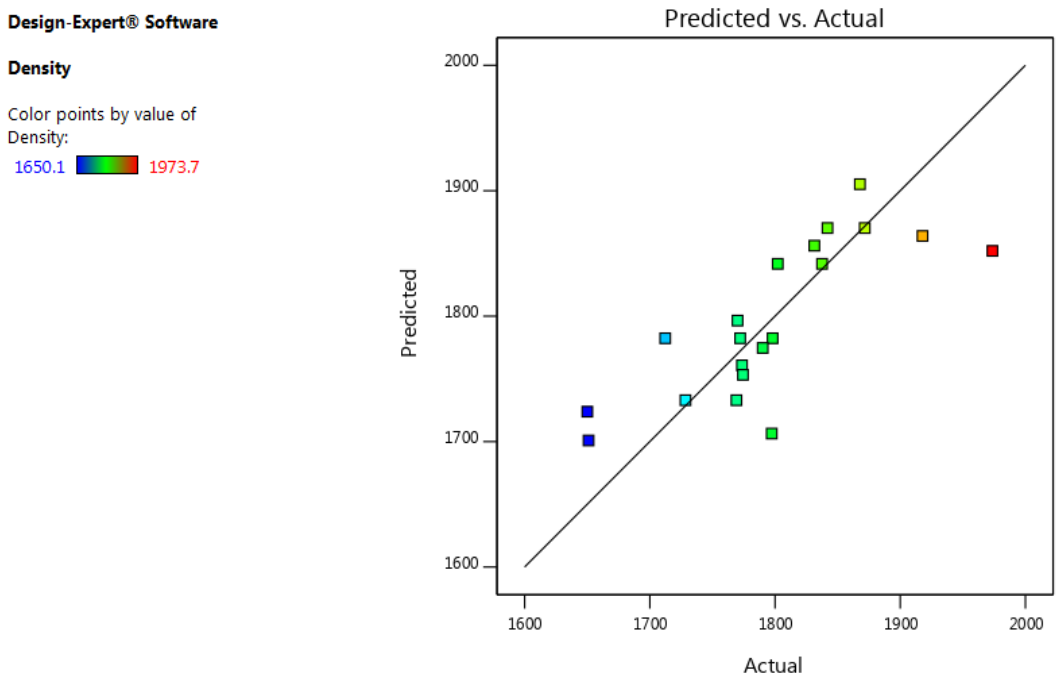


Figure 147: Scatter plot of the predicted values versus the actual values of the density response.

Trace plot in Figure 148 shows the behaviour of each component on the density. It is clear from the plot that, the behaviour of mango fibre quantity (D) is quite similar to its behaviour with the other three responses. On the other hand, the plot illustrates that the potato starch quantity (A) and mango starch quantity (B)

were in an inverse relationship with the density. While, the avocado starch quantity (C) was in direct relationship with the density. This means that, the density increases with the increasing of the avocado starch (C) and mango fibre (D) quantities and decreases with the increasing of any of the other two components.

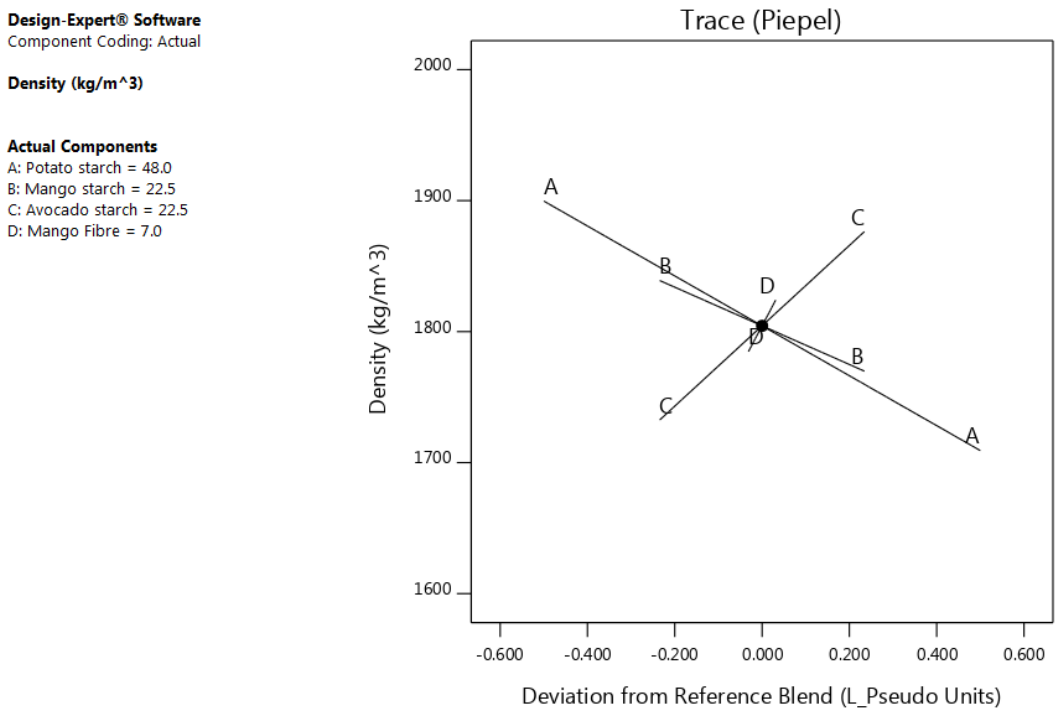


Figure 148: The Trace plot of the density response.

4.3.2 Starch And Fibre Homogeneity

As is mentioned in the previous chapter, 3-D optical microscope was used for evaluating the distribution of the starch and fibre in the bio-plastic sheet. Figure 149 illustrates the distribution of the starch and fibre on two different bio-plastic sheets. Looking at Figure 149, it can be noted that there are still an existence of large agglomerates of fibre despite the multiple mechanical pre-treatment which have been performed on the mango fibres. The figure also clearly showed that starch and fibre were not evenly distributed.

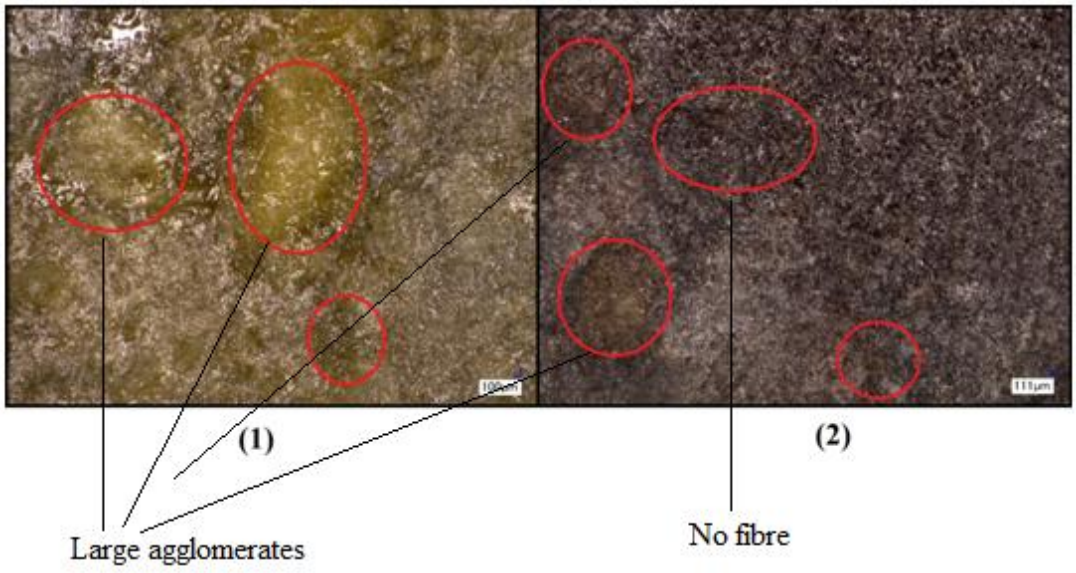


Figure 149: The distribution of the starch and fibre.

4.3.3 Bio-Plastic Sheet Process Key Findings

The mechanical properties tests and the density were carried out on the following day of the fabrication of the bio-plastic sheets. According to Table 37, the potato starch sheet was the best in terms of the mechanical properties, following by the avocado starch sheet. This finding is in line with what has been noted in literature regarding the excessive exploiting of traditional starch types in industrial applications [53, 306-308]. Due to that, un-traditional starch types such as; mango and avocado starch have not been extensively utilised in industries, etc. In addition to that, studies on the production of bio-products based on the them are quite low. As a result, it is not possible to make any comparisons between what has been reached in this study in terms of mechanical properties with others.

Furthermore, the tensile strength of the fibre-free potato bio-plastic sheet accomplished in this study is in accordance with the tensile strength of the bio-plastic sheet produced by Abdullah, A.H.D., et al. [309] after blending sweet potato starch with glycerine at a ratio of 3.5:1. When one compares between the tensile stress of the mango sheet and the tensile stress of mango starch bio-plastic sheet in a previous study [310], it can be noted that, the results from this study is lower by approximately 60%. Furthermore, another study on the production of the bio-plastic sheet from avocado starch [311], compared between the tensile strength of number of bio-plastic sheets, contained varied amounts of glycerine and reinforced materials. The study showed that, the tensile strength of the sheet, where it

contained starch to reinforced materials at a ratio of 9:1 and 30% glycerine of the starch weight of 1.05 MPa. This finding is close to the tensile strength of the fibre-free avocado sheet in this study. The presence of such differences in the mechanical properties may be attributed to the use of different cultivar of the same fruit, vegetables, or different types of plasticiser. Therefore, further studies are required to investigate the reasons for the presence of these differences.

The analysis of the results proved that, mango fibre has positive influence on the three studied mechanical properties and the density for the bio-plastic sheets. These influences were relatively low because the studied range of the mango fibre was low compared to the range of the total weight of the starch in the sheet. Therefore, it must be noted that when adjusting the rates of starch and fibre quantities, the volume of the bio-plastic sheet and the possibility of pressing the mould without bending or breaking it were taken into consideration. Moreover, due to the poor mechanical properties of the fibre-free mango starch bio-plastic sheet and the appearance of cracks on the sheet hours after its production, the range of the mango fibre was set to start from 4 g. This justification also enhances that the use of mango fibres to improve the mechanical properties of the sheets.

Looking at the analysis of the results, it can be noted that the quantity of the mango starch (B) has negative influence on all responses. The influences of the quantities of the potato (A) and avocado (C) starch were variable on the mechanical properties, while they were negative and positive on the density respectively.

The adequacy measuring tools for the impact loading strength and density responses were low. The farness from 1 are likely to be attributed to the use of an analogy scale when measuring the impact strength. While, it is attributed in case of the density to the uneven distribution of starch and fibre over the entire bio-plastic sheet and the existence of large agglomerates. Thus, further research into using a suitable mixing device for the blending of starch with the fibres could be used and extra filtration of the fibres could be done.

4.4 Summary Of The Key Findings

The chapter revealed some major findings, which can be summarised as follows:

- The residues of the potato, mango and avocado accounted for approximately 12.5%, 33% and 33% respectively of their total weight. Thus, exploiting of the wastes of the three biomass in the production of bio-products and by products would limit the quantity of their wastes which usually goes to landfill.
- The separated starch and mango coats do not have major positive influences on the biogas quantity and quality.
- Beating pre-treatment of the mango coats is not enough. Further pre-treatment is required before the beating pre-treatment to increase the accessibility of the enzymes to the biomass.
- Exploring more starch sources is required to reduce the pressure on the current available known sources of starch.
- Despite the great positive impacts of the digestate on the environment, its production costs may exceed its value.
- The examined digestate contains the basic nutrients of the conventional fertiliser in varied amounts and can be used in agriculture applications as another AD product. In order to fully assured the quality of it, conduction of further tests on the digestate is suggested.
- Increasing the dependence on digestate in agriculture applications or others, helps greatly reducing the costs are spending on it. This requires AD plants to increase farmers' awareness of digestate and the reliability on it
- All of the studied three factors of AD had varied influences on either the quantity and quality of the biogas produced or both. Organic concentration (B), sludge concentration (C) and their interaction (BC) had the most significant influences. Whereas maintaining a temperature of about 35 °C was seen as optimal. Hence, the levels of all factors must be carefully specified.
- In terms of the mechanical properties of the bio-plastic sheets; potato starch was the best following by avocado starch and mango starch.
- Mango seeds coats improved the mechanical properties and the density.
- Increasing the quantities of the avocado starch (C) and mango seed coats (D) increases the density.

- A suitable mixing device should be used to ensure a good homogeneity between starch and fibre. Also, extra filtration of the fibres should be carried out before blending it with the starch for ensuring the bio-plastic sheet produced is free of agglomerations.

Based on the above and as the production of the TPS/mango seed coats bio-plastic sheets does not required long processing or expensive materials and tools, its incorporation with the AD may help to enhance the economic viability of AD. The impact of the production process of the bio-plastic sheets on the economic aspects of the AD process is further investigated in the next chapter.

Chapter 5 : OPTIMISATION, ENERGY BALANCE AND COST EFFECTIVENESS

5.1 Introduction

This chapter is primarily aimed to investigate the influence of the incorporation of the production process of the TPS on the economic profitability of AD in a Lab scale. This was achieved by calculating the energy balance for the AD process and the cost effectiveness for the bio-plastic production process at optimum biogas yields and functional bio-plastic sheets in terms of the quality and costs. After setting the criteria in terms of quality and costs, the optimal biogas at their optimal set of factors were found. Thereafter, the energy balance was calculated for each of the three substrates (potato, mango and avocado) optimal biogas. This was conducted for each AD process of each biomass separately. Additionally, in determining optimal bio-plastic sheets in terms of the quality and cost, the cost effectiveness was calculated based on the bio-plastic sheets at their optimal combinations of starch and fibre, using the cost criterion. Figure 150 illustrates the structure of this chapter.

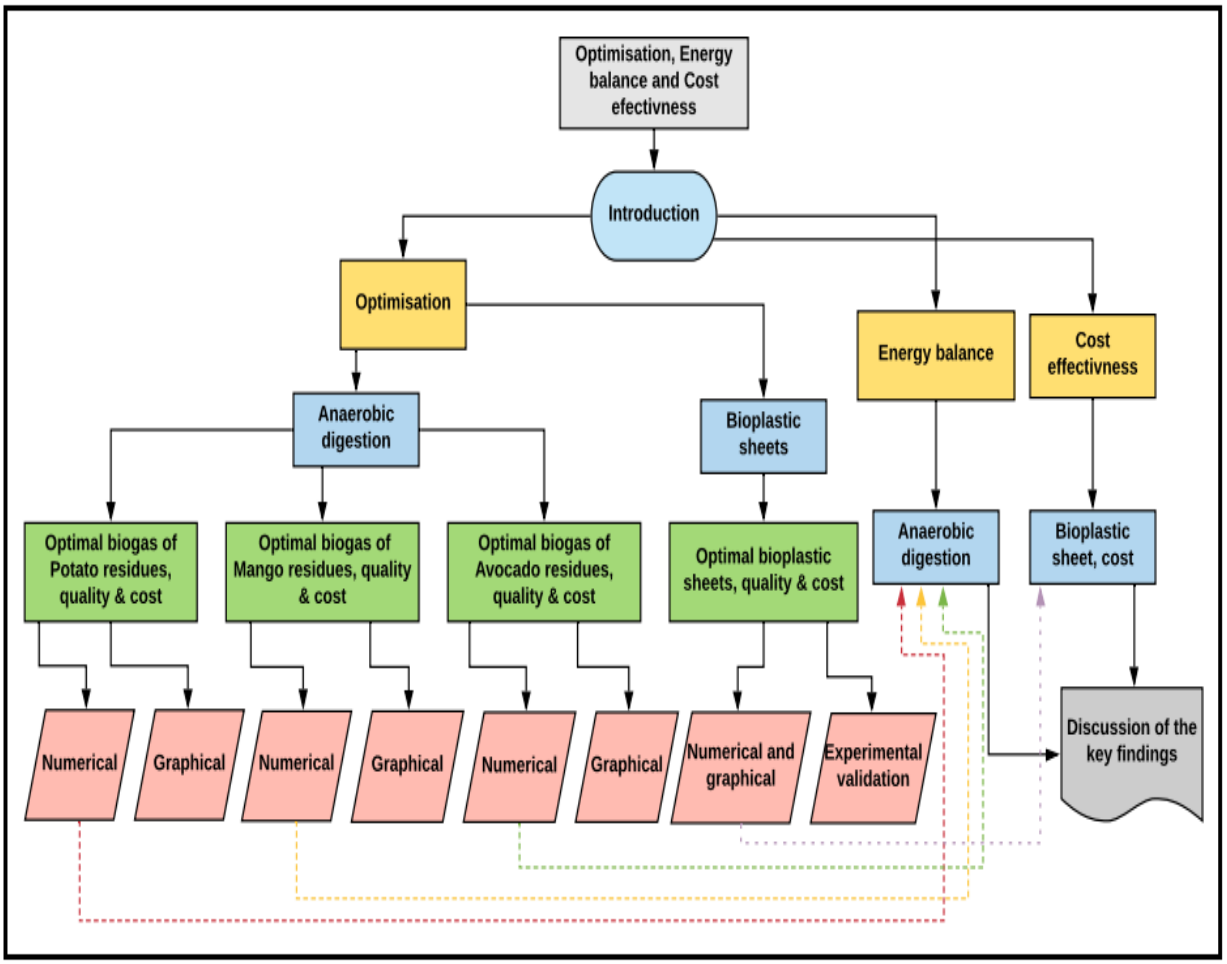


Figure 150: The structure of chapter 5.

5.2 Optimisation

This section optimises both processes to determine the optimal biogas and bio-plastic sheets at the optimal combinations of factors in terms of quality and cost. RSM, was used given the accuracy of its technique. It presents the optimal solutions numerically and graphically. The technique does so by searching for a combination of factor levels that simultaneously satisfy the requirements placed (i.e. optimisation criteria) on each one of the inputs/outputs. A mixture design technique was applied for optimising the properties of bio-plastic sheet and finding the optimal sheets in terms of quality and cost.

5.2.1 AD Processes Optimisation

In order to obtain an optimal solution in terms of the quality and cost for the results of the AD of each biomass, three optimisation criteria were set. The first criterion was in terms of the quality, aimed to find the optimal biogas for each biomass with no limitation on the process parameters at a minimum carbon dioxide concentration and maximum biogas yield/g-VS, methane concentration and the methane content. While, the second and third criteria were set to obtain the optimal biogas in terms of cost. As is mentioned in the previous chapter, the primary revenues of the AD plants are from gate fees, the sale of the heat and electricity and the sale of the digestate. Gate fees are not applied by every plant, as there are some plants that do not ask for fees to accept bio-wastes. On the other hand, the energy consumed in the pre-treatment of the biomass and digestion processes as well as the sludge fees are some of the major expenses for AD plants. Therefore, in the setting of the restrictions of the second criterion, the revenue of the AD plants from the sales of the electricity and the expenses on the energy consumed in the digestion process and sludge were taken into account. However, the energy consumed in the pre-treatment process of each of the biomass was neglected as it was quite low (0.15 kWh/5mins). The value of the digestate are quite low and sometimes AD plants give them away for free, therefore it was also neglected. Moreover, the same restrictions of the second criterion were set in the third criterion with a slight difference that, the gate fee as well as the total weight of VS (it is considered in the calculation of the electric energy consumed in the digestion process, E_c) were taken into account. In all criteria, the importance values of all responses were set at 3 except the methane concentration which was set at 5 (most important). The values of the methane

concentration and the biogas yield for each of the gram VS were both considered in the calculations of the energy balance. The preliminary attempts to find the optimum solutions when increasing the importance of the methane concentration and the biogas yield for each gram VS, showed that the difference between the solutions were very small. Due to that and to simplify the process, the importance value of the methane concentration was the only one set to 5. Table 42 shows the definitions three criteria.

Table 42: The three optimisation criteria of the AD process.

Name	Goals			Lower Limit	Upper Limit	Importance
	1st criterion	2nd criterion	3rd criterion			
Temp., °C	is in range	minimise	minimise	32	38	3
Organic Conc., g-VS	is in range	is in range	maximise	1.6	6.5	3
Sludge Conc., %	is in range	minimise	minimise	20	50	3
Total Biogas, cc	is in range	is in range	is in range			3
Biogas, cc/g-VS	maximise	maximise	maximise			3
Methane concentration, %	maximise	maximise	maximise			5
Carbon dioxide concentration, %	minimise	minimise	minimise			3
CH_4 content, cc/g-VS	maximise	maximise	maximise			3

1. Potato Residues

a. Numerical Optimisation

As per Table 43, the best biogas for the potato residues based on the first criterion was about 2286 cc, 1398 cc/g-VS, 72.4% CH_4, 22.1% CO_2 and 895 cc/g-VS CH_4. That was found at a 35 ^{0}C, 1.62 g-VS and 50% sludge. The table revealed that, the optimal biogas based on the second criterion was about 1501 cc, 791 cc/g-VS, 69% CH_4, 23.9% CO_2 and 574 cc/g-VS at the optimal condition of (34 ^{0}C, 1.83 g-VS and 38.9% sludge). Additionally, lower biogas in terms of biogas yield for each gram VS (cc/g-VS) were obtained by applying the third criterion at almost the same temperature and sludge concentration (34 ^{0}C, and 38.7% sludge) and 3.7 g-VS. Comparing between the optimum results based on the three criteria, it can be noted that the biogas obtained by the first criterion was remarkable the best. The optimal results support the discussion made earlier on the influence of the organic

concentration and sludge concentration and their interaction on the quantity and quality of potato residues biogas.

Table 43: Optimal solution of the AD of potato residues as obtained by DOE based on three criteria.

#	Temperature, °C (A)	Organic Conc., g-VS (B)	Sludge Conc., % (C)	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content, cc/g-VS
Optimal solution as obtained by DOE based on the 1st criterion								
1	35.01	1.62	50.00	2286	1397.89	72.44	22.13	895.39
2	35.02	1.62	50.00	2287	1397.82	72.45	22.13	895.25
3	35.02	1.62	50.00	2286	1397.94	72.45	22.13	895.37
Optimal solution as obtained by DOE based on the 2nd criterion								
1	34.06	1.83	38.94	1501	791.11	69.20	23.85	574.26
2	34.06	1.85	38.76	1503.52	785.19	69.20	23.77	569.06
Optimal solution as obtained by DOE based on the 3rd criterion.								
1	33.99	3.71	38.48	2207	593.53	69.20	19.78	402.22
2	34.00	3.69	38.38	2201	593.86	69.20	19.79	402.23
3	33.97	3.68	38.47	2198	595.00	69.20	19.77	403.39

b. Graphical Optimisation

An overlay plot represents the graphical optimisation. These plots are very helpful and practical. It greatly helps in identifying the optimal solutions and the optimal conditions quicker and at ease due to its graphical output. With graphical optimisation, the optimal range of each response determines the optimal range of results for each response derived from its numerical optimisation. Figure 151, Figure 152 and Figure 153, are the overlay plots (show the optimal solutions) for each criterion. In the plots, the green/shaded areas are the regions which meet the proposed criteria.

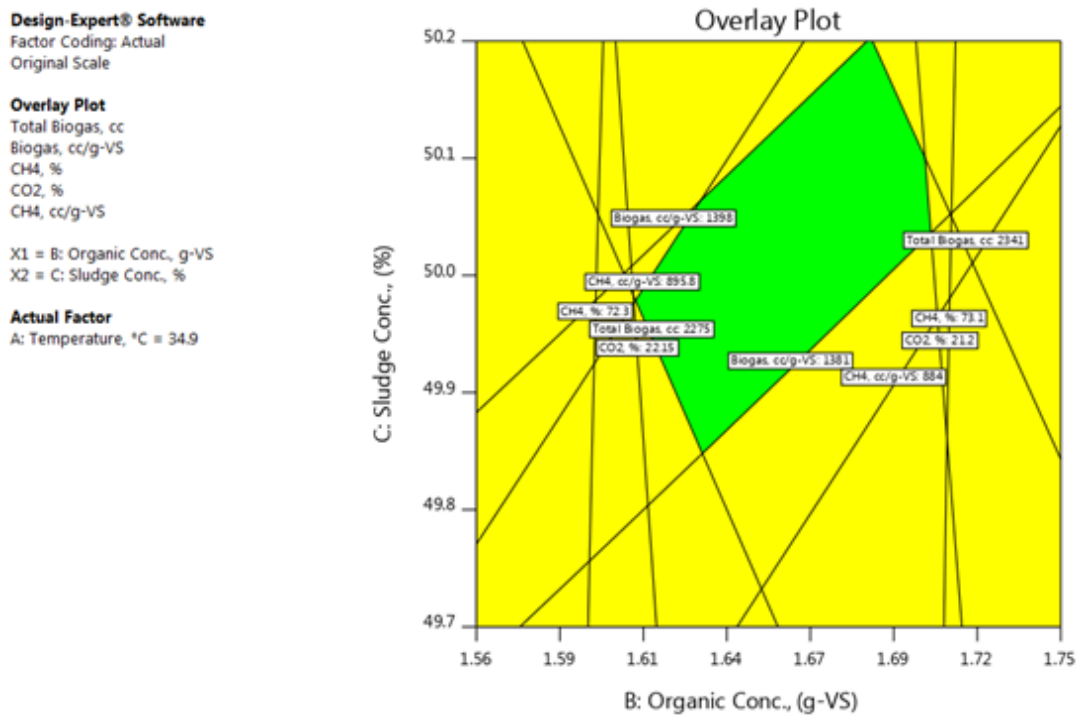


Figure 151: An overlay plot shows the optimum biogas based on the 1st criterion for potato.

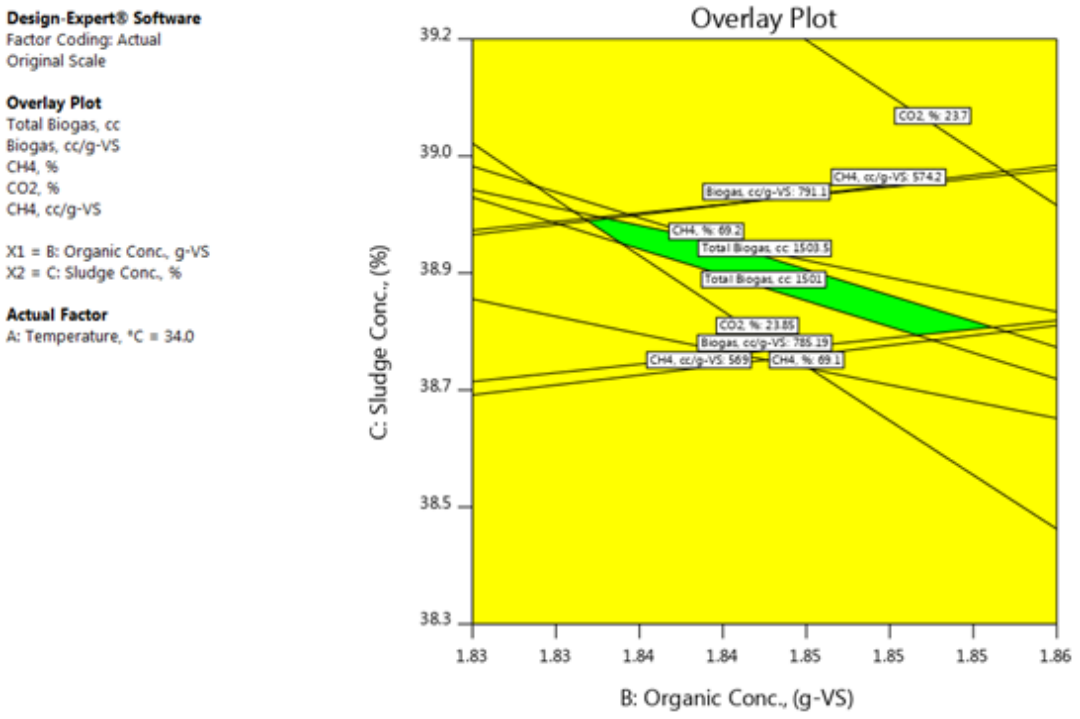


Figure 152: An overlay plot shows the optimum biogas based on the 2nd criterion for potato.

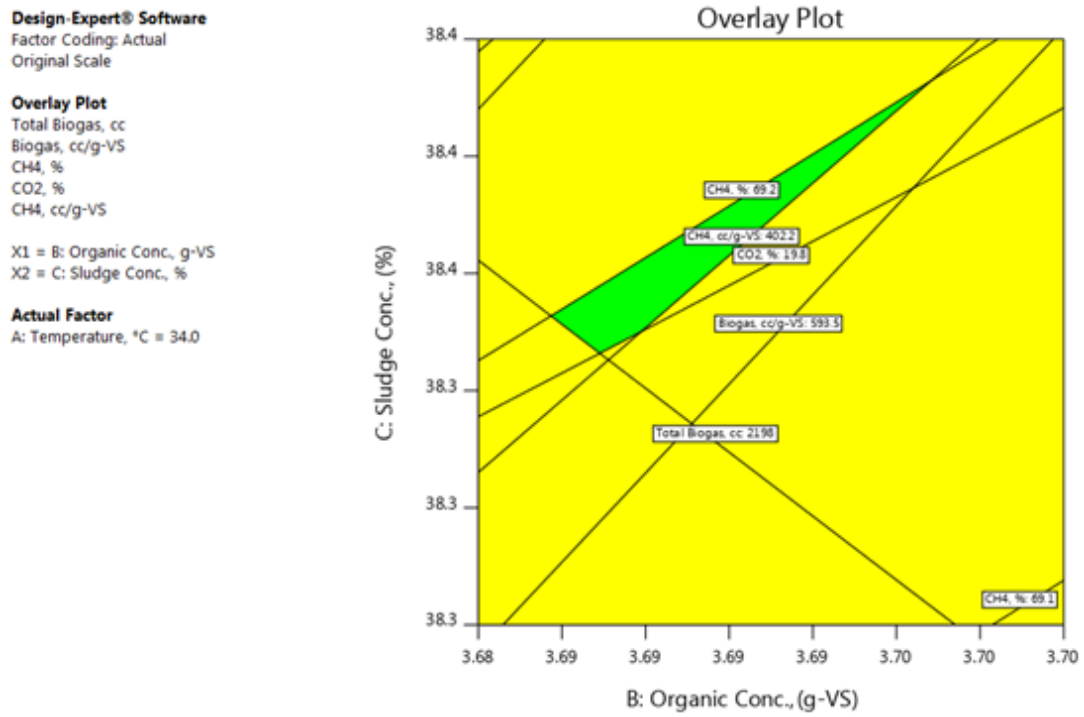


Figure 153: An overlay plot shows the optimum biogas based on the 3rd criterion for potato.

2. Mango Residues

a. Numerical Optimisation

According to Table 44, the optimal biogas of mango residues based on the first criterion was approximately 2830 cc, 805 cc/g-VS, 65.7 % CH_4, 24.4% CO_2 and 476 cc/g-VS CH_4. It was obtained at a biogas condition of 38 ^{0}C, 3.53 g-VS and 46% sludge concentration. Except for the total volume of biogas (cc), the optimal biogas of mango residues based on the second and third criteria were relatively closer to each other, but lower than the optimal biogas of the first criterion. They were found at conditions of (32 ^{0}C, 2.5 g-VS and 33% sludge) and (32^{0}C, 3.9 g-VS and 37 % sludge) respectively. As is evident from Table 44, the changing of the goal of the temperature parameter in the second and third criteria to "minimise", resulted in a descent of the optimum temperature from its highest level to the lowest level (32 ^{0}C). This indicated that, the temperature does not make significant changes on the responses.

Table 44: Optimal solution of the AD of mango residues as obtained by DOE based on the three criteria.

#	Temperature, °C (A)	Organic Conc., g-VS (B)	Sludge Conc., % (C)	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content, cc/g-VS
Optimal solution based on the first criterion								
1	38.00	3.53	46.00	2825	804.88	65.66	24.41	475.86
2	38.00	3.53	46.07	2831	806.11	65.61	24.44	476.36
3	38.00	3.51	45.82	2801	803.16	65.76	24.38	475.14
Optimal solution based on the second criterion.								
1	32.00	2.51	33.36	1394	561.96	60.33	27.99	302.24
2	32.00	2.51	33.27	1389	560.94	60.31	28.00	301.57
3	32.00	2.52	33.49	1403	563.02	60.37	27.98	303.09
Optimal solution based on the third criterion								
1	32.00	3.93	37.02	1976	510.59	62.49	27.41	293.28
2	32.00	3.93	37.12	1982	512.08	62.49	27.40	294.04
3	32.00	3.95	37.08	1984	509.97	62.47	27.43	292.96

b. Graphical Optimisation

Looking at the analysis of the results of mango residues and the three overlay plots in Figure 154, Figure 155 and Figure 156, it can be noted that these findings are in line with what have been discussed earlier in the analysis regarding to the influence of each factors on the biogas yield and quality. It is evident however from the numerical and graphical optimisations of mango residues and the analysis, that the influences of the organic concentration and sludge concentration were more significant than the influence of the temperature on all responses.

Design-Expert® Software
Factor Coding: Actual

Overlay Plot
Total Biogas, cc
Biogas, cc/g-VS
CH4, %
CO2, %
CH4, cc/g-VS

X1 = B: Organic Conc., g-VS
X2 = C: Sludge Conc., %

Actual Factor
A: Temperature, °C = 38.0

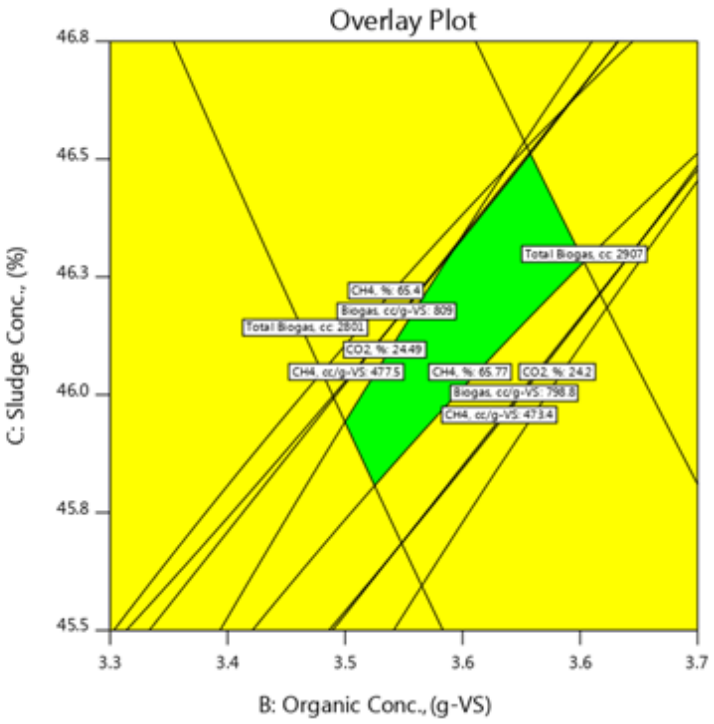


Figure 154: An overlay plot shows the optimum biogas based on the 1st criterion for mango.

Design-Expert® Software
Factor Coding: Actual

Overlay Plot
Total Biogas, cc
Biogas, cc/g-VS
CH4, %
CO2, %
CH4, cc/g-VS

X1 = B: Organic Conc., g-VS
X2 = C: Sludge Conc., %

Actual Factor
A: Temperature, °C = 32.0

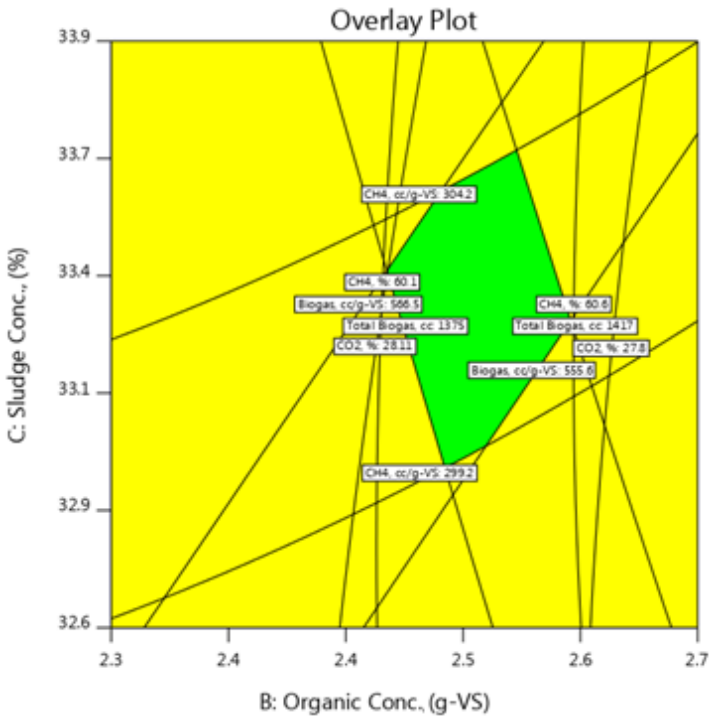


Figure 155: An overlay plot shows the optimum biogas based on the 2nd criterion for mango.

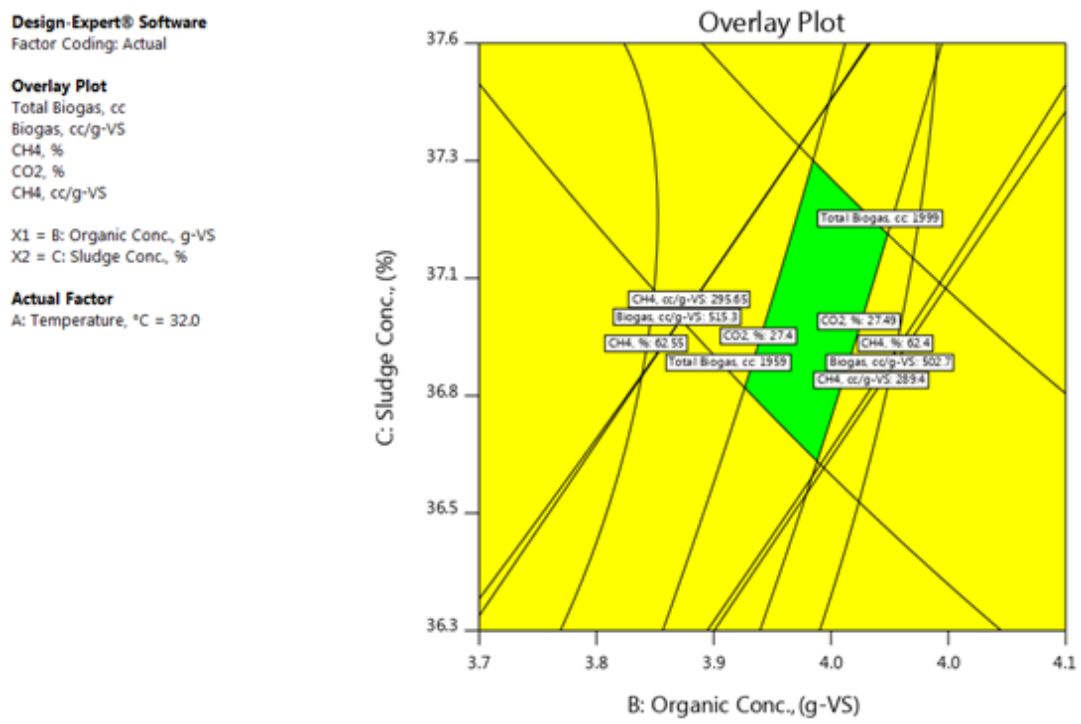


Figure 156: An overlay plot shows the optimum biogas based on the 3rd criterion for mango.

3. Avocado Residues

a. Numerical Optimisation

Table 45 shows the numerical optimisation of the avocado residues based on the three criteria. The table clearly shows that, the optimal biogas was located at a temperature of close to 35 ºC. This can be observed, as when the goal of temperature was changed, the optimal temperature variation in the three tables was slight. On the other hand, the changes of the goals of the three parameters indicated that, the optimal avocado residues biogas based on the three criteria were located between 1.6 -1.9 g-VS and 40-42 % sludge. It worth noting that, when the goal of the organic concentration was "is in range", the optimal was at 1.6 g-VS in the optimum results of the first and second criterions. However, when it was changed to "maximise", the optimal solutions were located at 1.9 g-VS. This increase in the organic concentration led to a minor increase in the total volume of the biogas (cc).

Table 45: Optimal solution of the AD of avocado residues as obtained by DOE based on the three criteria.

#	Temperature, °C (A)	Organic Conc., g-VS (B)	Sludge Conc., % (C)	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content, cc/g-VS
Optimal solution as obtained by DOE based on the 1st criterion								
1	35.27	1.60	40.70	1683	1046.96	61.30	22.03	610.58
2	35.26	1.60	40.82	1686	1048.66	61.26	22.06	611.28
3	35.29	1.60	40.71	1684	1047.52	61.28	22.03	610.81
Optimal solution as obtained by DOE based on the 2nd criterion								
1	34.93	1.60	39.88	1650	1028.50	61.69	21.83	602.20
2	34.92	1.60	40.00	1652	1030.22	61.65	21.86	602.89
3	34.95	1.60	39.97	1653	1030.21	61.66	21.84	603.01
Optimal solution as obtained by DOE based on the 3rd criterion.								
1	35.17	1.92	41.84	1869	1007.05	60.96	21.93	586.89
2	35.58	1.92	41.48	1877	1009.81	60.87	21.84	588.31

b. Graphical Optimisation

Figure 157, Figure 158 and Figure 159, are the graphical optimisation of the avocado residues based on the three criteria, which support what was discussed in the numerical optimisation of the avocado residues. Figure 159 shows that the optimal region of AD of avocado residues based on the third criterion, was very limited.

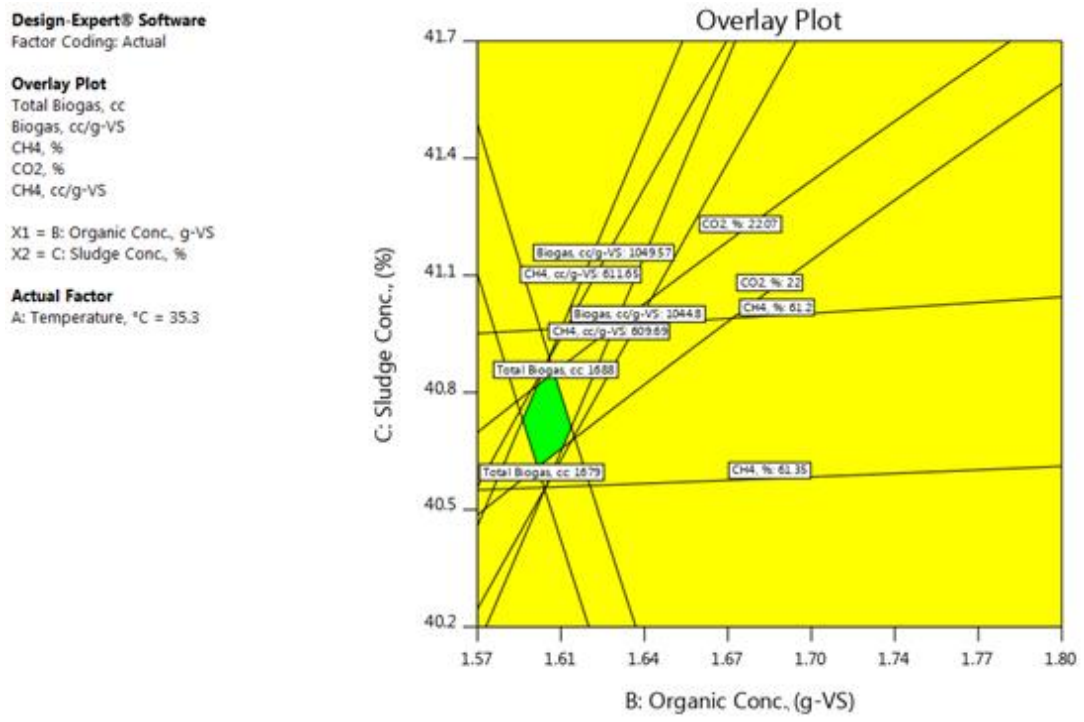


Figure 157: An overlay plot shows the region of the optimal avocado residues biogas based on the 1st criterion.

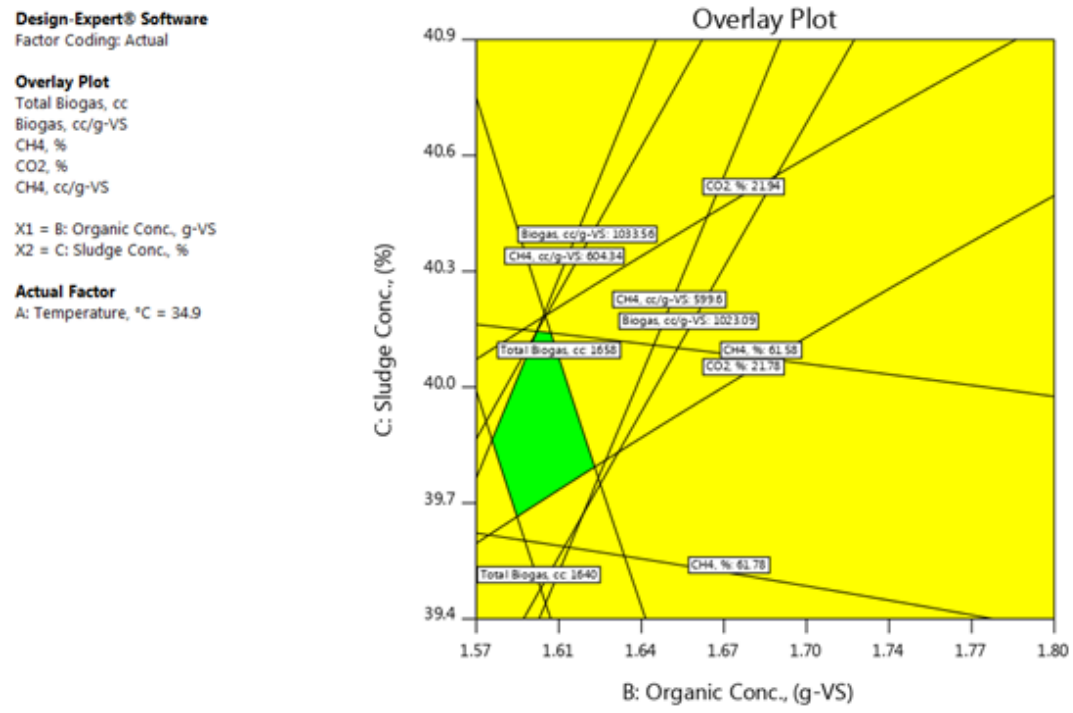


Figure 158: An overlay plot shows the region of the optimal avocado residues biogas based on the 2nd criterion.

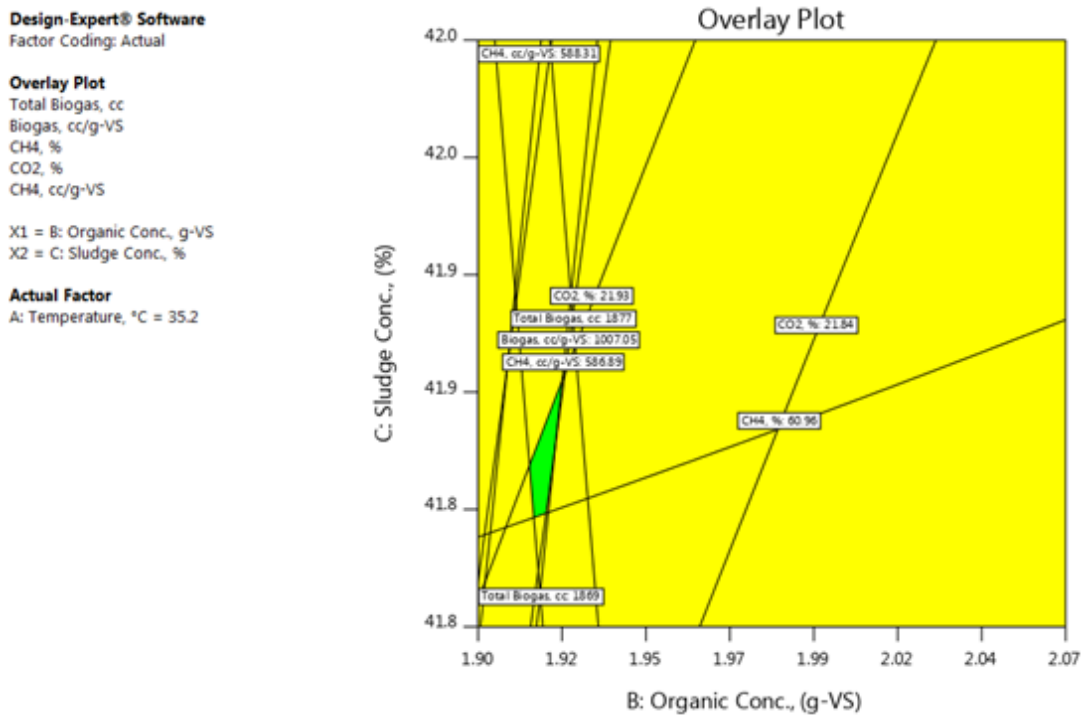


Figure 159: An overlay plot shows the region of the optimal avocado residues biogas based on the 3rd criterion

5.2.2 Bio-Plastic Sheets Production Optimisation

In order to obtain an optimal bio-plastic sheet, in terms of the quality and cost as a result of the bio-plastic production process, two optimisation criteria were

applied. The first criterion was in terms of the quality, aimed to find the bio-plastic sheet with no limitation on the process parameters at the maximum of all responses. The second criterion was in terms of cost. In the setting of the restriction of the second criterion, each of the four ingredients were set to maximum in order to find the optimal mixture of them. The importance values of all responses were set at 3 with the exception of the density which was set at 5 (most important). This was because the cost of the bio-plastic will be calculated later in this chapter by its density. Table 46 shows the two criteria.

Table 46: The two optimisation criteria of the bio-plastic production process.

Name	Lower Limit	Upper Limit	Goal	Importance	Goal	Importance
			1st criterion		2nd criterion	
A:Potato starch	0	96	is in range	3	maximise	3
B:Mango starch	0	45	is in range	3	maximise	3
C:Avocado starch	0	45	is in range	3	maximise	3
D:Mango fibre	4	10	is in range	3	maximise	3
Tensile strength			maximise	3	maximise	3
Flexural stiffness			maximise	3	maximise	3
Impact strength			maximise	3	maximise	3
Density			maximise	3	maximise	5

a. Numerical and Graphical Optimisation

Table 47 is the numerical optimisation of the bio-plastic sheets based on the two criteria. The table shows that, the optimal quantity of the starch and fibre at the optimal bio-plastic sheet in terms of quality was located at A= 90 g, B=0 g, C= 0 g and D= 10 g. On the other hand, the changes of the goals of the four ingredients to maximise and the importance of the density to 5 resulted in an optimal mixture of A= 47.06 g, B= 7.66 g, C= 35.3 g and D= 10 g. Figure 160 and Figure 161 are the graphical optimisation of the bio-plastic sheet production process based on the two criteria. They are supporting the finding of the numerical optimisation. It worth noting that, when the goals of ingredients were set to maximise and the importance of the density to 5, the quantities of the mango and avocado starch increased. This finding motivates for utilising mango and avocado starch in industrial applications or others.

Table 47: Optimal solution of the bio-plastic sheets production process based on the two criteria.

Potato starch, g	Mango starch, g	Avocado starch, g	Mango Fibre, g	Tensile strength, MPa	Flexural stiffness, MPa	Impact strength, J/cm^2	Density, kg/m^3
Optimal solution as obtained by DOE based on the 1st criterion [Quality]							
90	0	0	10	2.22	2.70	18.47	1753.24
Optimal solution as obtained by DOE based on the 2nd criterion [Cost]							
47.06	7.66	35.3	10.0	1.46	3.08	13.99	1873.14

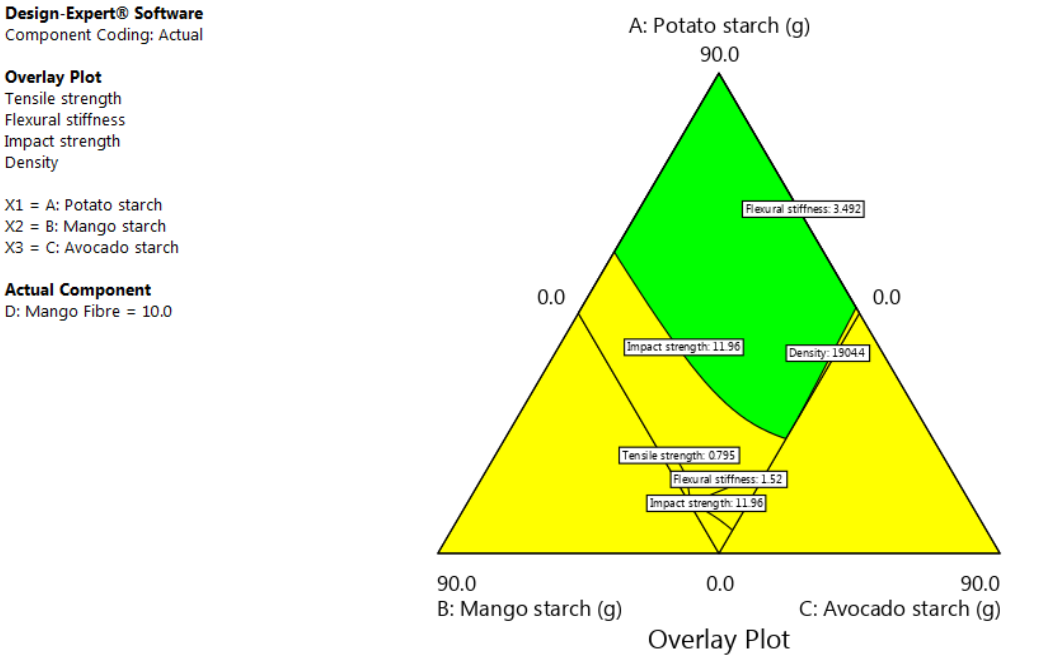


Figure 160: An overlay plot shows the region of the optimal bio-plastic mixture based on quality (1st criterion).

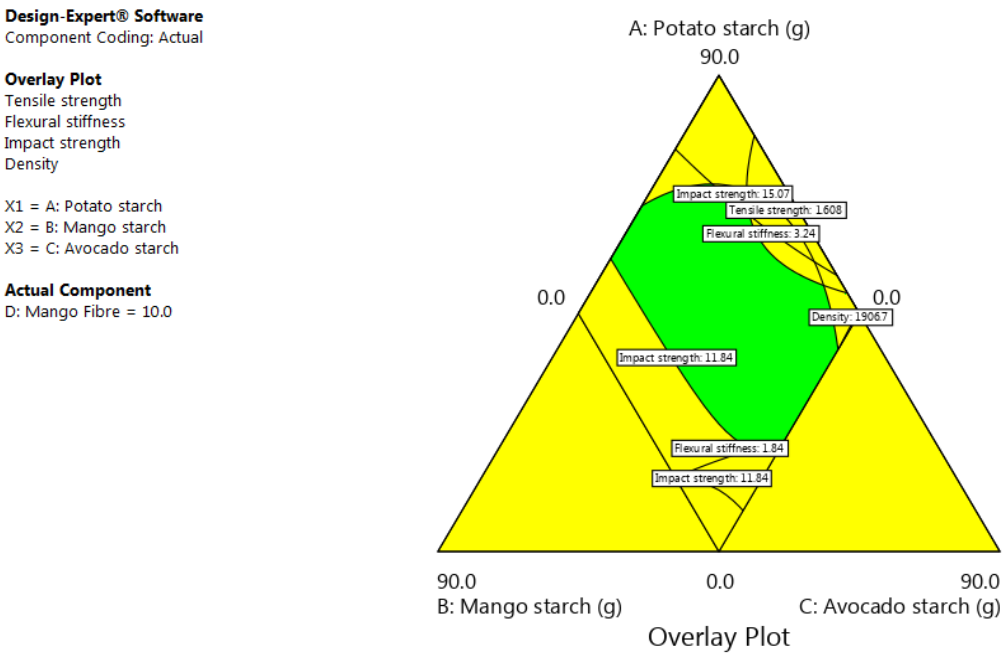


Figure 161: An overlay plot shows the region of the optimal bio-plastic mixture based on cost (2nd criterion).

b. Experimental validation of the optimal results

In order to validate the developed mixture model and the optimal results, a bio-plastic sheet was produced using the ingredients obtained from optimal results based on the cost criterion as shown in Figure 162. Different specimens were produced by cutting the bio-plastic sheet as described in Chapter 3. The four responses of interest were measured according to the procedures mentioned in Chapter 3. Table 48 shows the error percentages between the experimentally measured responses (Actual) and the responses from optimal setting (Predicted) of the bio-plastic shown in Table 47. In Table 48, it is evident that the maximum error percentage was 8.2%, which was for the tensile strength, whereas the lowest was 0.5%, for the impact strength. Overall according to Table 48, it can be said that the bio-plastic model and the optimal results are valid as all the error percentages were in reasonable agreement. Moreover, to evaluate the cost effectiveness of the production process of the bio-plastic sheet, the aim is to optimise the process to produce a bio-plastic sheet has the same ingredients and properties of the bio-plastic sheet based on the cost criterion and therefore evaluate the influence of the incorporation of the bio-plastic production process with the AD on the economic aspects of the AD. In order to validate the cost based on mechanical performance, the related physical property influencing cost is density. Therefore as the density

error in Table 48 accounts for only 0.57%, it can be assumed that the predicted cost was not affected significantly when comparing the validated predicted/actual results.

Figure 162: The optimal bio-plastic sheet produced based on the cost criterion.

Table 48: Validation of the optimal result.

Optimal setting					Tensile strength, MPa	Flexural stiffness, MPa	Impact strength, J/cm^2	Density , kg/m^3
Potato starch, g	Mango starch, g	Avocado starch, g	Mango Fibre, g					
47.06	7.66	35.3	10.0	Predicted	1.46	3.08	13.99	1873.14
				Actual	1.59	3.24	13.92	1883.93
				Error %	8.2	4.94	0.50	0.57

5.3 Energy Balance

The energy balance of each of the three AD processes were found out by calculating the difference between the energy consumed and the energy produced. The energy consumed in the digestion process were the only expense considered in the calculations of the energy balance of each AD of the biomass. As the electric energies consumed in the beating pre-treatment were low, they were neglected. While, the energy content of the biogas produced were the only revenue considered. Gate fees is not applied by all AD plants and there is no standard pricing scheme for it. While, the value of the digestate is very low and again there is no standard pricing scheme for it. Therefore, the revenues from the gate fees and digestate were disregarded in the calculations of the energy balance.

The electric energies consumed by the water baths at each of the three temperature levels in the digestion processes were measured by electric consumption meters. The graph in Figure 163 presents the average electric energies consumed at each temperature's level throughout each AD experiment (21 days). As is clear from the graph, the average energies consumed at the 32, 35 and 38º C were 41.33, 54.75 and 83.25 kWh respectively.

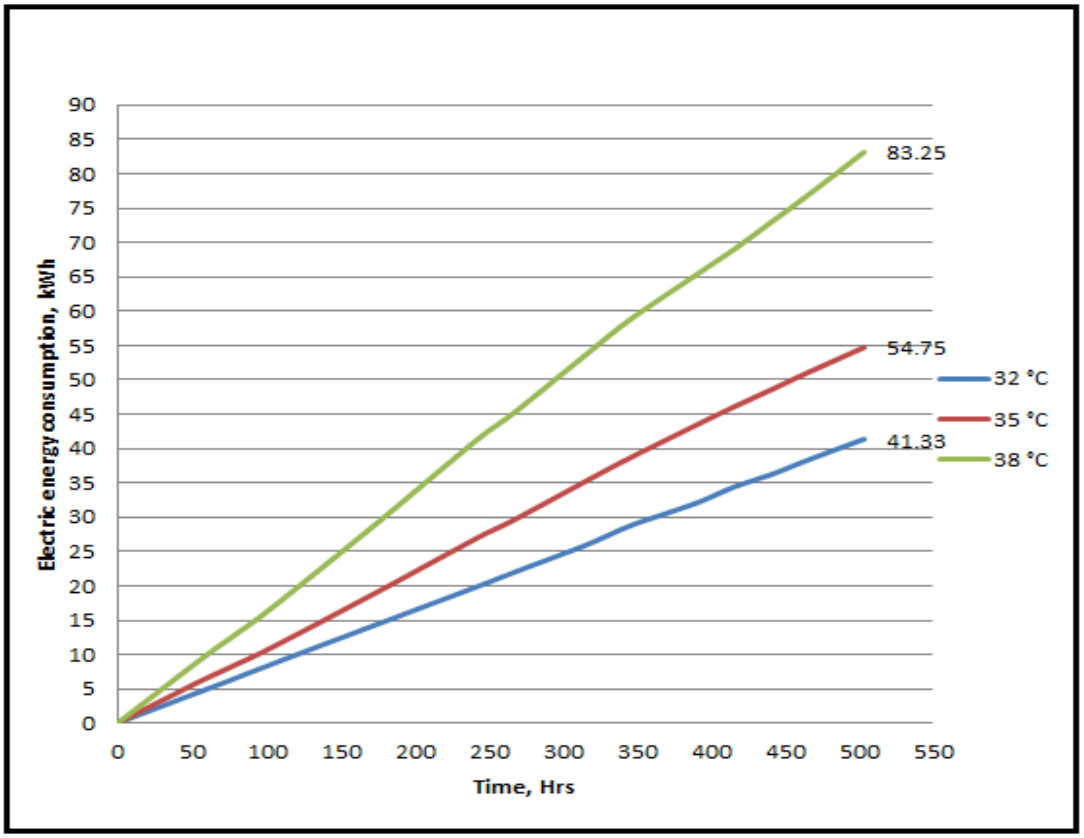


Figure 163: The average electric energies consumed at each temperature's level.

During AD, the digestion processes were carried out in water baths. The total capacity of each water bath was not fully utilised. Whereas, only 12 flasks were placed in each water bath and the baths were running at full performance at the pre-specified temperature levels. According to the water bath's manual, the dimensions of each water bath is 590 x 220 x 350 mm and its full capacity is 45 liters. The electric energy which was consumed by each water bath at each of the three temperature levels in Figure 163 are based on using them at full capacity. Which means that, whether there was only one reactor or 12 in the water bath, the energy consumption remained the same. Therefore, a number of assumptions were set, are;

1) The full capacity of the water bath was exploited.
2) Only one reactor with a volume of 2/3 of the total volume of the water bath was used. This one reactor was equivalent to the volume of 75 reactors of the reactors have been used in the experiment (=30 L).
3) The remaining capacity (1/3) was the volume of the water used to heat up the reactor in the bath.

In order to calculate the energy balance for each AD process of biomass at each of the three criterion in Table 42, the selected optimum results by the RSM technique were the ones considered in the calculations. Table 49 shows the selected optimal results and their conditions.

Table 49: The optimum results which were selected by RSM.

Biomass	Criterion	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	Total biogas vol., cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 Content, cc/g-VS
Potato	1st	35.01	1.62	50.00	2286	1397.89	72.44	22.13	895.4
	2nd	34.06	1.83	38.94	1501	791.11	69.2	23.85	574.3
	3rd	33.99	3.71	38.48	2207	593.53	69.2	19.78	402.2
Mango	1st	38.0	3.53	46.00	2825	804.88	65.66	24.41	475.9
	2nd	32.0	2.51	33.36	1394	561.96	60.33	27.99	302.2
	3rd	32.0	3.93	37.02	1976	510.59	62.49	27.41	293.3
Avocado	1st	35.27	1.60	40.7	1683	1046.96	61.3	22.03	610.6
	2nd	34.93	1.60	39.88	1650	1028.5	61.69	21.83	602.2
	3rd	35.17	1.92	41.84	1869	1007.05	60.96	21.93	586.9

Table 50, presents the energy balance of the AD of each biomass based on each optimisation criterion. According to the three assumptions, the optimum volume of biogas produced from each gram of VS of the biomass (B_p) as well as the total weight of the VS (VS_m) were multiplied by the assumed number of reactors (75).

Table 50: The energy balance of the AD of each biomass based on each optimisation criterion.

Biomass	Criterion	Energy consumed, kWh	VS weight, g	B_s, kWh/m^3	E_p, kWh/g-VS	E_c, kWh/g-VS	Net E_p, kWh/g-VS	Energy gain/loss, %
Potato	1st	54.8	1.62	7.00	0.73	0.45	0.284	62.9%
	2nd	53.3	1.83	6.69	0.40	0.39	0.009	2.3%
	3rd	53.2	3.71	6.69	0.30	0.19	0.107	55.9%
Mango	1st	83.3	3.53	6.35	0.38	0.31	0.069	21.9%
	2nd	41.3	2.51	5.83	0.25	0.22	0.026	12.0%
	3rd	41.3	3.93	6.04	0.23	0.14	0.091	65.0%
Avocado	1st	55.2	1.60	5.93	0.47	0.46	0.006	1.2%
	2nd	54.6	1.60	5.97	0.46	0.46	0.005	1.1%
	3rd	55.0	1.92	5.89	0.45	0.38	0.063	16.5%

As is evident from Table 50, the highest energy gain by the one gram volatile solids of potato residues, reached at 62.9% at 35 ^{0}C, 1.62 g-VS and 50% sludge,

which according to Table 49 would yield maximum CH_4% of 72.44%. In other words, based on the three criteria, the production of the biogas from one gram of volatile solid of potato residues was at its highest level at 35 ^{0}C, 1.62 g-VS and 50% sludge, i.e. 62.9% higher than the energy consumed in its production. The results of the energy gain/loss from one of gram volatile solid of mango residue revealed that, the highest energy gain in terms of the cost was 65% and about 22% when the quality was taken into consideration (Table 50). Regarding to the highest energy gain produced by one gram volatile solids of the three biomass, it can be said that the highest energy gain achieved was 65% by one gram volatile solid of mango residues based on the 3rd criterion. In addition, the highest energy loss was attributed to the one gram volatile solids of avocado residues based on the 2nd criterion. It was achieved at the condition of 34.9 ^{0}C, 1.6 g-VS and 39.88% sludge concentration, which yielded a maximum methane concentration of 61.69% according to Table 49.

In reference to Table 50, it can be observed that changing the goal of the organic matter concentration to "maximise", caused the energy gain to increase remarkably in all biomass. In contrast, when the goals of the temperature and sludge concentration were set to "minimise", large losses in the energy balance of potato and mango residues were noticed while, the energy balance of avocado was almost the same.

It is worth mentioning that, the highest energy gains of the mango and avocado residues were achieved based on the third criterion, which yielded a maximum methane concentration of 62.49 % and 60.96 % (65% and 16.5% Table 50) respectively. While, the highest energy gain from the potato residues was in accordance with the 1st criterion, which yielded a maximum CH_4% of 72.44 %. Looking at Table 50, it can be observed that, the highest energy gain obtained from the potato residues were at the same criterion which yielded the highest methane concentration, while the highest energy gains obtained from mango and avocado residues were not at the same criteria which yielded the highest methane concentrations. These can be attributed to that the energy consumed by the water bath for digesting the mango residues at the 1st criterion (which yielded the highest methane concentration) was approximately double the energy consumed based on

the 3rd criterion (at the highest energy gain acquired). It worth noting that, in the calculation of the energy consumed (E_c), the energy consumed by the water bath is divided by organic concentration and multiple by 75. Additionally, the organic concentration at the 3rd criterion (where the highest energy gain achieved) was the highest compared to the organic concentrations obtained based on the 1st and 2nd criteria. Hence, the energy consumed (E_c) based on the 1st and 2nd criteria were higher than the energy consumed by based on the 3rd criterion. In regards to the optimal results of the avocado residues, the organic concentration based on the 3rd criterion was the highest compared to the other two, therefore the highest energy gain was achieved based on the 3rd criterion.

As is previously mentioned in more details, the current study was primarily aimed to enhance the profitability of the AD process to make it more desirable for investment. From the key findings of the energy balance of the AD of the three biomass based on the quality criterion (1st criterion), it can be concluded that, at the same constraints, influencing factors and their levels, the production of biogas from one gram of organic potato waste was the highest among other biomass. In terms of cost, the highest energy gain acquired was from the optimal result of the mango residues based on the 3rd criterion. Despite that the highest biogas volume per one gram volatile solids and methane concentration were resulted from the potato residues based on the 1st criterion of potato, the highest energy gain was resulted from the mango residues based on the 3rd criterion. This can be attributed to the energy consumed (E_c) and the amount of the organic concentration, as the organic concentration at the optimum result of mango residues based on the 3rd criterion (3.93 g-VS) was much higher than the organic concentration at the optimum result of the potato residues based on the 1st criterion (1.62 g-VS).

In addition, Table 50 also revealed that, at almost the same optimum temperature and sludge concentration (2nd and 3rd criteria of potato and avocado residues), the increasing of the organic matter of potato residues and avocado residues by approximately 103% and 20% respectively led to a major increasing in the energy balance. Another large increase in the energy balance was noticed when the organic matter weight of the mango residues was increased by 56% at almost the same temperature and a 11% increase of sludge concentration (2nd and 3rd criteria of

mango residues). As previously mentioned, one of the reasons for setting the 3rd criterion in this form is the application of gate fees by some AD plants. These key findings of the large increase in the energy balance when increasing the organic matter enhance AD plants for applying gate fees for accepting wastes, allowing for processing larger amount of wastes and therefore, increase the contribution of AD in waste management.

5.4 Cost Effectiveness Of The Production Of The Bio-Plastic Sample Sheet

Two optimisation criteria were implemented as mentioned previously. The goals of the two criteria were to find the optimal mixtures used to produce the bio-plastic sample sheets with dimensions of 150x150x3.2 mm^3 in terms of its quality and cost. In this section, as cost effectiveness of the sample sheet is to be assessed, the optimal mixture of the bio-plastic sample sheet in terms of its cost was the one which considered in the calculation. The optimal quantity of each ingredient are as follows: potato starch 47.06g, mango starch 7.66, avocado starch 35.3 and mango fibre 10g. The following sub-headings describe the cost effectiveness calculation;

1. Energy Consumed

The energy consumed in separating and/or treating the ingredients to form the optimal bio-plastic sample sheet are presented in Table 51 below. The potato separation process was not considered, because it was included in the calculation of the energy balance of the AD process.

Table 51: The energy consumed in the production of the optimal bio-plastic sample.

#	Process	Energy consumed, kWh	Energy consumed, kWh/sample
1	Mango starch separation by the Hollander Beater	0.0005357/g	0.00410
2	Avocado starch separation by the Hollander Beater	0.0002778/g	0.00980
3	Separation of large agglomerates of fibres	0.011/g	0.11
4	Pre-treatment of fibre by NaOH for 2 hrs	0.00077/g	0.0077
5	Mechanical pre-treatment of fibre	0.0004615/g	0.00461
6	Heating of the sample sheet during production *.	0.43/g	0.43
	Total	0.4430	0.566

* Assuming the energy consumed for producing one gram is equal to energy consumed for producing a sample sheet.

2. ***Bio-Plastic Sheets Estimated Cost***

The total energy consumed in producing one bio-plastic sample sheet was approximately 0.566 kWh. Table 52 shows the estimated expenses for the production of the optimal bio-plastic sample sheet based on the cost criterion.

Table 52: The estimated expenses for the production of the optimal bio-plastic sample sheet.

#	The estimated cost of producing an optimal bio-plastic sample sheet	Cost, €
1	Energy consumed as per the average unit price in DCU *	0.07077
2	Glycerine [312]	0.0855
3	Sodium hydroxide [312]	0.0133
4	Sodium meta-bisulfite (2g mixed with 200 ml distilled water in 2 separate beakers)	0.015
	Total	0.184/ sample sheet (134.86 g)

*According to the DCU Estate office, the DCU power consumption rate is 0.125 euro.

Table 52 reveals the estimated total cost for producing one of the optimal bio-plastic sample sheet is €0.184. This indicated that, the cost for producing one gram of the optimal bio-plastic sample sheet is equivalent to € 0.00136/g ($\frac{€\ 0.184}{134.86\ g}$). The above calculations were calculated on a laboratory scale sample. In order to be more realistic, these calculations should be generalised over one of the standard industrial dimensions of plastic. Assuming that, the dimension of the standard industrial dimension bio-plastic which would be produced is 2440*1220*3.2 mm^3 [313]. That is approximately 132.3 times bigger than the bio-plastic sample sheet used in this study. Also, the calculation was carried out on the assumption that, the standard industrial bio-plastic sheet has exactly the same properties and density of the bio-plastic sample sheet as shown in Table 53. Therefore, Table 54 shows the estimated expenses for producing a standard industrial bio-plastic sheet, where the total cost for producing the bio-plastic standard industrial sheet is approximately €34.53.

Table 53: The specifications of the bio-plastic sheet has the selected standard dimension.

Dimension	2440*1220*3.2
Mass	17.84 Kg
Volume	0.00952 m^3
Density	1873.14 Kg/m^3
Quantity of each components	Potato starch=6226.03 + mango starch = 1013.41 + avocado starch = 4670.19 g+ mango fibre= 1323 (total weight of all components = 13230g)
Quantity of glycerol	3572.1 g
Quantity of NaOH	661.5 g
Quantity of meta sulphite	264.6

Table 54: The estimated expenses for the production of the TPS sheet has the selected standard dimension.

	Expense	Cost, €
1	Operating cost/ standard sheet	24.43
2	Hourly labour wages in Ireland in 2020 [314]	10.10
	Total cost	34.53

3. Bio-Plastic Sheets Sales

The price of the fossil-based plastic mainly depends on oil prices and fluctuates with the oil indices while, the price of bio-plastic is more stable. On a weight basis, bio-based plastic is more expensive than fossil-based plastic which can be attributed to the higher density of the bio-based plastic [300]. Wageningen Food and Bio-based Research (WFBR) specified the cost of a bio-plastic by its density. According to WFBR in 2016 [300], the price of a starch blend bio-plastic material with a density between 1250 and 1350 kg/m^3 ranged from 2 to 4 €/ kg [300]. As the density of optimal sample sheet is about 1873.14 kg/m^3, which is higher than 1350 kg/m^3, then the bio-plastic produced in this study would be at least €4/kg. According to this, the price of bio-plastic standard industrial sheet is approximately €71.36. Therefore, the production of the standard dimensional bio-plastic sheet based on the specifications listed in Table 53, is cost-efficient by approximately 51.6%.

By assuming that the price of bio-plastic would remain to increase in a linear mode with the density beyond 4 €/kg. Then the price of the bio-plastic produced in this study would be approximately €5.55/kg. According to this, the price of bio-

plastic industrial standard sheet is approximately €99.01. Therefore, the production of the standard dimensional bio-plastic sheet based on specifications listed in Table 53, is cost-efficient by approximately 65.1%.

In industries, power consumption rates are usually less as the voltage is higher (380V, 3 phase). In addition, the cost effectiveness was calculated based on the power consumption rate for Dublin City University. Certainly, by calculating it based on the industrial rate this would lead to a higher cost efficiency. Also, the labour would produce more sheet per hour, which would reduce the production cost per sheet.

The hydraulic press, the mould and the cutting machine are one-time large purchases assets (fixed-cost). In order to calculate the annual profitability of the production of the starch-based bio-plastic sheets and the estimated period required for the AD plants or others to start reaping profit, the total costs of the one-time purchases items should be subtracted from the profits until balanced-budget is achieved.

These findings are not beneficial only for AD plants, some other industrial and commercial sectors can also benefit from them (i.e. fruits and vegetable wholesale markets). Such markets can gain profits by simply producing their bio-plastic containers out of their waste. As they might be losing money in disposing their waste. In the meantime, they will still need to buy plastic containers from an external supplier to pack their products. Thus, by fabricating the bio-plastic containers for use and/or sale would significantly reduce the waste. This would result in decreasing the disposal charges and add a new profitable by-product. Additionally, these findings also encourage fruits and vegetables markets for separating the starch from the wastes of their products and selling it to the industries as raw materials.

5.5 Chapter Summary

In this chapter, the optimal AD conditions at which the maximum biogas for each biomass have been found and presented. Also, the energy balance of each biomass was calculated based on the optimal AD conditions. Table 55 below shows the highest energy gains reached at the optimal setting for each of the three biomass. On the other hand, the optimum bio-plastic mixtures of the starch of the three biomass and mango fibre were also found in terms of quality and cost. Thereafter, the cost efficiency of bio-plastics production process was calculated based on the optimal results of the cost criterion.

Table 55: The optimum results at the highest energy gains reached for each of three biomass.

Biomass	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	Total biogas vol., cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 Content, cc/g-VS	Energy gain/loss, %
Potato	35.01	1.62	50.00	2286	1397.89	72.44	22.13	895.4	62.9%
Mango	32.0	3.93	37.02	1976	510.59	62.49	27.41	293.3	65.0%
Avocado	35.17	1.92	41.84	1869	1007.05	60.96	21.93	586.9	16.5%

Table 56 illustrates the total weight of the waste resulting from each biomass, the weight of the starch and mango fibre separated. The weights were estimated based on the weights measured for each of the three biomass, starch and fibre in the previous chapter.

Table 56: The estimated weight of total wastes, starch and fibres.

Biomass	The weight per one fruit/veg., g	Total weight of waste per one fruit/veg., g	Starch weight extracted, processed and ready to use per one fruit/veg., g	The weight of the fibre separated, processed and ready to use/ one mango, g
Potato	125	15.62	2.2	
Mango	407	145	5	6
Avocado	223	70.62	6	

In order to attain the following two goals: 1) the highest energy gain for each biomass at the optimal setting, and 2) reaching a cost efficiency of 65.1% by producing an industrial standard bio-plastic sheet that has the same properties and density of the optimal bio-plastic sample sheet, the following waste quantities are

required: (i) 6.226 kg of potato starch, which required a total potato peels weight of 44.20 kg, this would lead to 35.67 kg VS as presented in Chapter 4. (ii) 1.013 kg of mango starch, which required a total mango waste of 31.972 kg, this would lead to 28.46 kg VS. (iii) 4.670 kg of avocado starch, which required a total avocado waste of 54.94 kg, this would lead to 47.78 kg VS. Table 57 illustrates the quantity of starch required from each biomass to attain the two goals, it also shows the total biogas volume which would be produced from each biomass by the AD process based on these amounts of waste.

Table 57: The quantity of the starch required to be processed and the total biogas would be produced.

#	Biomass	Starch quantity required, kg	Total weight of the waste required, kg	VS weight, kg	Total biogas, L
1	Potato	6.226	44.204	35.67	49858.63
2	Mango	1.013	31.972*	28.46	14531.80
3	Avocado	4.670	54.943**	47.78	48121.33

*(19845.05294 g peels +5512.51g seeds (1102.502 g starch) + 6615 g coats (1323g fibres)).
**(28687.2681 g peels + 26256.1437 g seed)

In summary, to produce a total biogas from each biomass according to the volumes outlined in Table 57 and a single standard industrial bio-plastic sheet, approximately 2830 potatoes (353.75 kg), 221 mangoes (89.94 kg) and 779 (173.71 kg) avocados are required to be processed separately. Certainly, the exploitation of this amount of waste in the production of biogas and bio-products would greatly contribute in reducing the diversion of these amounts of wastes to landfill and would serve to turn such losses in to financial profit for any fruit/vegetable distributer.

Figure 164 shows a simplified diagram of the proposed integrated approach in this study. It is a completed life cycle, started with the growing of the three fruits and vegetables in a farm and ended up with the production of the AD biogas, TPS bio-plastic sheets and bio-fertiliser. Table 58 shows the total energy produced and the total energy consumed of each of the three highest energy gains in Table 55 based on the production of the standard industrial dimension bio-plastic sheet. Figure 165 shows a diagram summarising the major findings of the study based on the proposed integrated approach in Figure 164. As it is clear from the figure, the remaining bio-energy is approximately 15658.1 kWh, which is approximately 29%

of the bio-energy produced. In reference to the proposed integrated approach in Figure 164, the inputs in the integrated approach are the crops of the three biomass and the outputs are the three bio-products produced. According to the life cycle of the bio-plastic in Figure 4, after disposing of a biodegradable bio-plastic, the organic waste will be composted and returned to the earth to help new crops grow. On the other hand, the digestate generated post to AD can be further used as bio-fertiliser, while the remaining bio-energy (29% of the bio-energy produced) can also be used to power the plant, therefore the cycle is completed.

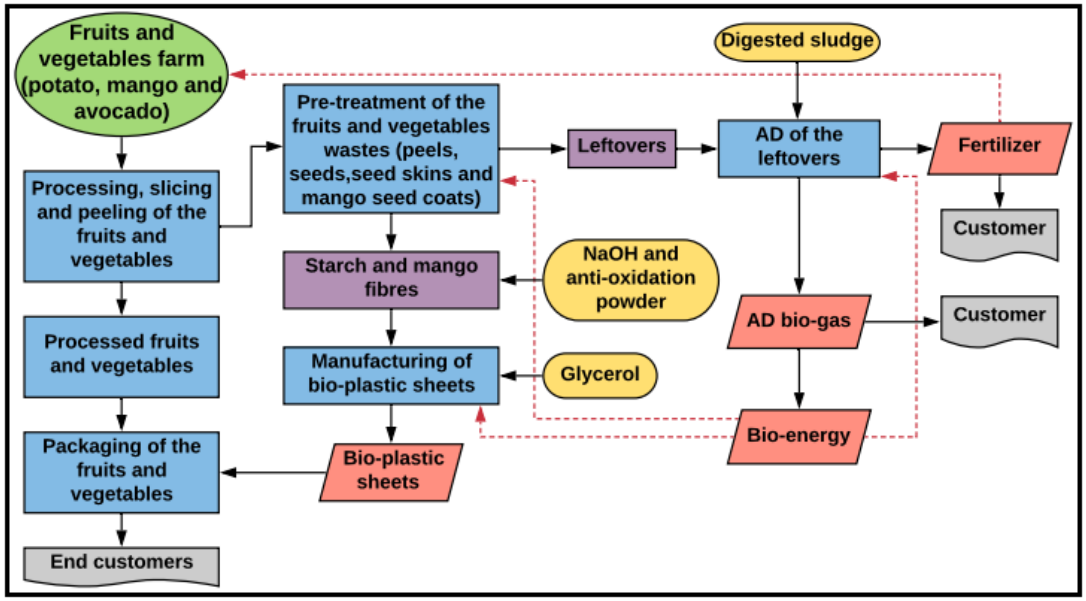


Figure 164: A simplified diagram of the proposed integrated approach.

Table 58: Total energy consumed and produced at each of the three highest energy gain at the optimal biogas of each of three biomass.

	E_p, kWh/g-VS	E_c, kWh/g-VS	VS, g	Energy produced, kWh	Energy consumed, kWh	Energy gain, %
Potato	0.73	0.45	35667.06	26194.28	16075.13	62.9
Mango	0.23	0.14	28460.79	6585.94	3990.79	65.0
Avocado	0.44	0.38	47784.45	21275.04	18256.29	16.5
Total				54055.25	38322.21	

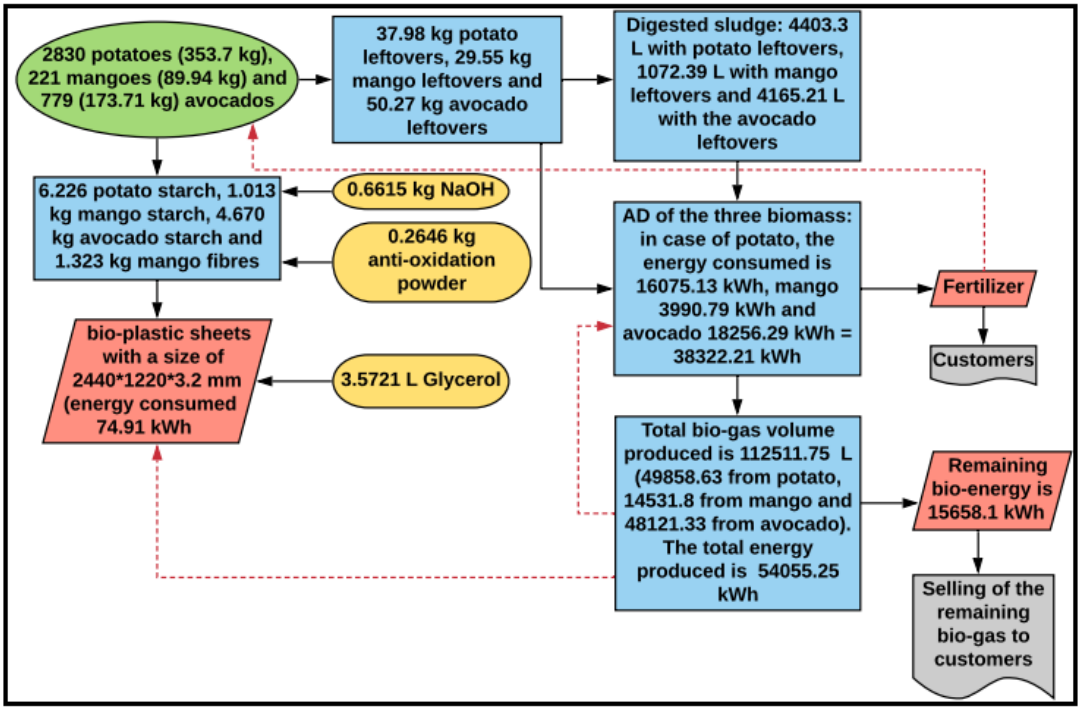


Figure 165: A summary of the major findings of the study.

Chapter 6 : CONCLUSION AND FUTURE WORK

6.1 Conclusions

This section is further divided into sub-headings to summarise the main conclusions as well as the future research and limitations (where exist) of the anaerobic digestion process, bio-plastic production process and the bio-energy produced.

6.1.1 Anaerobic Digestion

a. Main Conclusions

- The exploitation of the large amounts of waste generated from potato, mango and avocado in the production of bio-energy and bio-products would increase the contribution of the AD in waste management.
- The amounts of the substrate and sludge must be carefully balanced inside the digesters.
- The starch from the three biomass and mango coats do not significantly influence biogas quantity and quality, therefore incorporating them in the production of the bio-plastic sheets along with the AD process or using the starch as raw material for producing other starch based bio-products, exploits their use.
- The significance of the influence of temperature was higher on the quality of the biogas of avocado residues than its influence on the quantity of the biogas produced from avocado residues and even higher than its influence on the quantity and quality of the biogas produced from the other two biomass.
- The highest energy gain by one gram volatile solids of potato residues was 62.9% at 35 ^{0}C, 1.62 g-VS organic concentration and 50% sludge concentration, which yielded a maximum CH_4% of 72.4%. On the other hand, the highest energy gain by one gram volatile solids of mango and avocado residues were approximately 65% and 16.5% which yielded a maximum CH_4% of 62.49% and 60.9 respectively.
- The study has achieved the bio-refinery concept which aims to make full use of biomass through using the whole of the three biomass in producing bio-energy and bio-products
- The results obtained revealed that the study was able to reduce the digestate resulting from the AD by confirming that the digestate contains the basic nutrients of fertiliser and urging for more tests to increase the reliability of using the digestate as organic fertiliser.

- ❖ The results of the incorporation of the two process confirms the potential of this incorporation on the economic aspects of the AD process.
- ❖ In addition to the AD plants which could limit or even overcome the challenges associated with the AD by applying the proposed integrated approach, fruits and vegetables wholesale companies could also increase their profits by applying such an approach.
- ❖ Knowing the obstacles and challenges before embarking on any project helps well in the success of the project. Therefore, this study can greatly help countries such as the Kingdom of Saudi Arabia as one of the largest countries in producing and exporting oil which has recently started the investment in renewable energy to diverse the revenues and reduce the environmental impacts of the fossil fuel in achieving excellence and prosperity in the AD field.

b. Requires Future Research

- ❖ Increasing the dependence on digestate in agriculture applications or other uses, helps greatly in reducing the costs that are spent on processing it. To achieve this, AD plants must increase farmers' awareness of the benefit of digestate and their reliability on it. This can be reached by subjecting the digestate to more tests to measure its contents of other elements.
- ❖ The results of the energy balance stimulate AD plants to apply gate fees for accepting wastes. Which, would increase the profitability of the AD process.

c. Limitations

- ❖ The employment of the Hollander Beater for pre-treating mango seed coats is not sufficient. They require further pre-treatment to increase the accessible surface area and size of the pores available for hydrolytic enzymes. Alternatively, it can be utilised in waste-to-energy plants or in other industrial applications such as; bio-filler, bio-fibre, etc.

6.1.2 Bio-Plastic production

a. Main Conclusions

- ❖ The results reached proved the potentials of potato, mango and avocado starch as raw materials of bio-plastics. This motivates for further exploration of more starch sources to reduce the pressure on the available ones and substitute the fossil fuel-based materials by biomaterial-based products.
- ❖ In terms of the mechanical properties of the bio-plastic sheets, potato starch was the best followed by avocado starch and mango starch.

- Mango fibres improve the mechanical properties and the density of the bio-plastic sheets.
- The increasing in the quantities of the mango fibres and avocado starch accompanied by an increasing in the density of the bio-plastic sheets.
- Production of a bio-plastic sheet with a dimension of 2440*1220*3.2 mm has the same specifications of the optimal bio-plastic sheet produced could result in a cost efficiency up to 65.1%.
- In order to produce a bio-plastic sheet according to the proposed integrated approach with a dimension of 2440*1220*3.2 mm, 353.7 kg potato, 89.94 kg mango and 173.71 kg avocado are required to be processed.

b. Limitations and Future Research

- A suitable mixing device for blending the starch with the fibres should be used and extra filtration of the fibres should be done.

6.1.3 Bio-Energy

a. Main Conclusions

- Implementation of the proposed approach could result in an excess amounts of bio-energy equals approximately to 29% of the bio-energy produced.
- The remaining bio-energy produced can be sold separately as heat or electricity to increase the profitability or, used to power the plant, which can further reduce the expenses of AD plant.
- Producing bio-energy and bio-products via applying the suggested integrated approach in this study by an AD plant or others will stimulate other AD plants or even countries to take this approach.

b. Limitations

- The present study was carried out on a laboratory scale. The application of the proposed integrated approach in this study by AD plants and others requires them to implement it firstly on a large scale.

6.2 Future work

- ❖ The contents of the digestate to other elements such as: total humic and fulvic acid and heavy metals (Zn, Cu, Sn, Cd, Ni, Pb, Hg, Cr) should be measured to further assure the quality and validity of the digestate. The digestate content of each element should then be compared to the permissible ratio for the element.
- ❖ Producing of glycerine out of the avocado oil, use it as a plasticiser in the production of the bio-plastic sheets and compare the mechanical properties of the sheets produced with resulted mechanical properties of the sheet produced in this study. This requires investigation on the quantity and quality of the biogas produced from the avocado wastes before and after the separation of the oil from the waste. This helps in making full use of the biomass and increases the number of bio-products produced to improve the economic aspects of the AD.
- ❖ Evaluate the impacts of the starch extracted from other unconventional sources of starch (eg. peas) on the quantity and quality of the biogas produced from the wastes of these sources in order to reduce the pressure on the available common sources of starch.
- ❖ Continuous measurement of the volume of AD biogas produced in order to monitor the production of the biogas and calculate the production rate.
- ❖ Strengthen the calibration procedures of the meters and analyser used.

6.3 Research Contribution

The following statements, identify how this research work contributes to a Doctor of Philosophy, along with the list of publications given at the front of the thesis:

- No studies were found investigating the influence of the incorporation of the bio-plastic production process with the AD on the environmental and economic aspects of AD.
- It is the first study to produce bio-plastic sheets along with the products of the AD in an integrated approach.
- No studies were found evaluating the quantity and quality of the AD biogas produced from a biomass before and after separating of starch.

- Beating pre-treatment has been applied previously prior to the AD in multiple studies for pre-treating of several types of biomass such as: Irish seaweeds. This is the first study that has been applied beating pre-treatment on mango and avocado residues, and use the Hollander Beater as a multi-functional device, that for: pre-treating the biomass mechanically, isolating starch and processing mango seed coats.
- Only a few studies were found on mango and avocado starch. Mango and avocado starch have not yet been exploited in industrial applications.
- New knowledge was obtained by using mango coats as reinforced materials as no studies were found using it as reinforced materials. This knowledge obtained lies in knowing its influence on the mechanical properties of the bio-plastic sheets and its potentials as a reinforcement material.
- New results obtained from blending the three starch with each other in producing bio-plastic sheets or even blending two of them together as no studies were found combining them together in producing bio-plastic sheets.
- A unique knowledge was acquired regarding the calculation of the energy balance of the AD digestion process and the cost efficiency of the bio-plastic production process. Whereas, the calculations of the energy balance and the cost efficiency have been carried out based on assumptions have been set to facilitate the evaluation of the economic feasibility of the integrated approach.
- It is the first study has used the DOE in analysing the AD process of the mango and avocado residues and the production process of the bio-plastic sheets (using potato, mango, avocado and mango fibres) and optimising them.

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APPENDICES

Appendix A : The Three Biomass Used

Figure 166: Russet potato.

Figure 167: The potato peels.

Figure 168: The mango and its residues, a) mango seeds, b) mango peels, c) mango fruit and, d) mango seed coats.

Figure 169: Pre-treated seed coat fibres.

Figure 170: Fuerte avocado, a) avocado fruit b) avocado peels c) avocado cut seeds, and d) avocado seeds.

Appendix B : The Certificates Obtained From The Sludge Supplier On The Sludge and Liquid Digestate And The Ones Awarded To The Plant.

ENVIRONMENTAL CHEMISTRY TEST CERTIFICATE

Report Status: **Replacement Report**
Date of Issue: **11-Dec-2017**
Report Number: **432180**
Project: **1-171124-09951**
Page 1 of 1

This report replaces Report Number: **431143**

Attention: **Robbie Holohan**
Client: **Green Generation Ltd**
Address: Nurney
Co. Kildare

Order Number:

Disclaimer
Results in this report relate only to the items tested.
Reports may not be reproduced in full without the approval of **Advanced** Laboratory **Testing**.
Results reported as cfu/cm2 are calculated based on information supplied by the relevant customer regarding the specific area swabbed.
* beside the method or lack of INAB symbol signifies that **Advanced** Laboratory **Testing** are not INAB accredited for this method.
Samples are retained post analysis for a period of 10 days. Samples are stored frozen by default except in the case of M&S and customer requirements.

ALT ID: 1077952 **Date Received:** 24/11/2017 **Date Tested:** 01/12/2017
INAB P9 Classification: Others: Digestate
Client ID: Liquid Digestate

Test	Result	Unit(s)	Method	Technique
Total Phosphorous	1611	mg/kg	ECTM008	HACH Method 8190
Potassium	5248	mg/kg	ECTM023	In-House
Dry Matter	5.6	g/100g	ECTM024	In-House
Total Nitrogen	7105	mg/kg	ECTM025	In-House

Figure 171: The certificate obtained from the AD plant for the results of the tests of the liquid digestate.

ENVIRONMENTAL CHEMISTRY TEST CERTIFICATE

Report Status: **Final Report**
Date of Issue: **01-Feb-2018**
Report Number: **449804**
Project: **1-180116-06080**
Page 1 of 1

Attention: **Robbie Holohan**
Client: **Green Generation Ltd**
Address: Nurney
Co. Kildare

Order Number:

Disclaimer
Results in this report relate only to the items tested.
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Results reported as cfu/cm2 are calculated based on information supplied by the relevant customer regarding the specific area swabbed.
* beside the method or lack of INAB symbol signifies that **Advanced** Laboratory **Testing** are not INAB accredited for this method.
Samples are retained post analysis for a period of 10 days. Samples are stored frozen by default except in the case of M&S and customer requirements.

Project Comment: Results reported are based on the sample as received

ALT ID: 1125634 **Date Received:** 16/01/2018 **Date Tested:** 19/01/2018
INAB P9 Classification: Sewage
Client ID: Sludge

Test	Result	Unit(s)	Method	Technique
Total Phosphorous	1371	mg/kg	ECTM008	HACH Method 8190
Potassium	2168	mg/kg	ECTM023	In-House
Dry Matter	5.9	g/100g	ECTM024	In-House
Total Nitrogen	7844	mg/kg	ECTM025	In-House

Figure 172: The certificate obtained from the AD plant for the results of the tests of the sludge.

Figure 173: The sludge, company and certificates.

Appendix C : The Drawings Of The Design Of The Mould

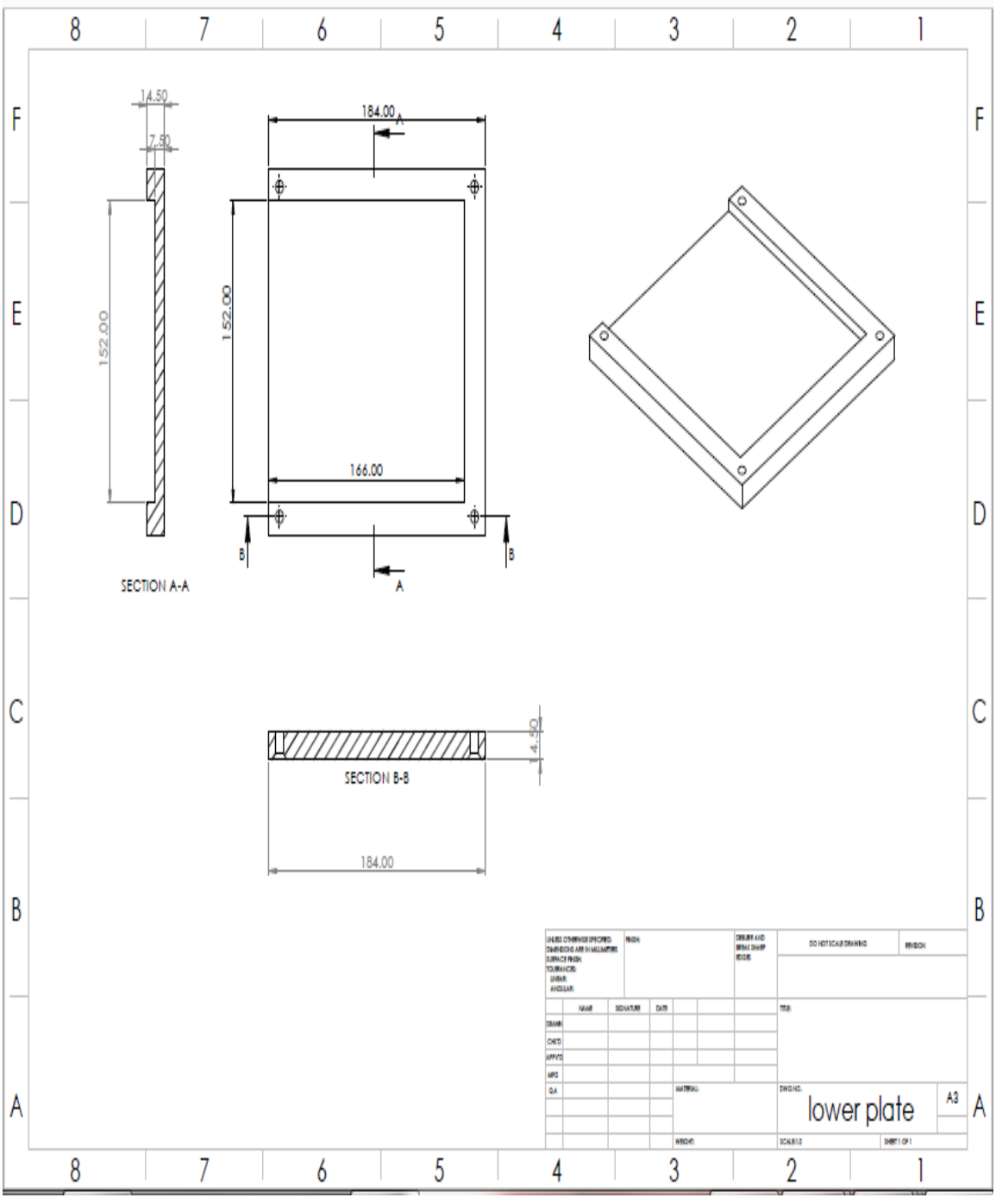

Figure 174: The design of the bottom plate of the lower part.

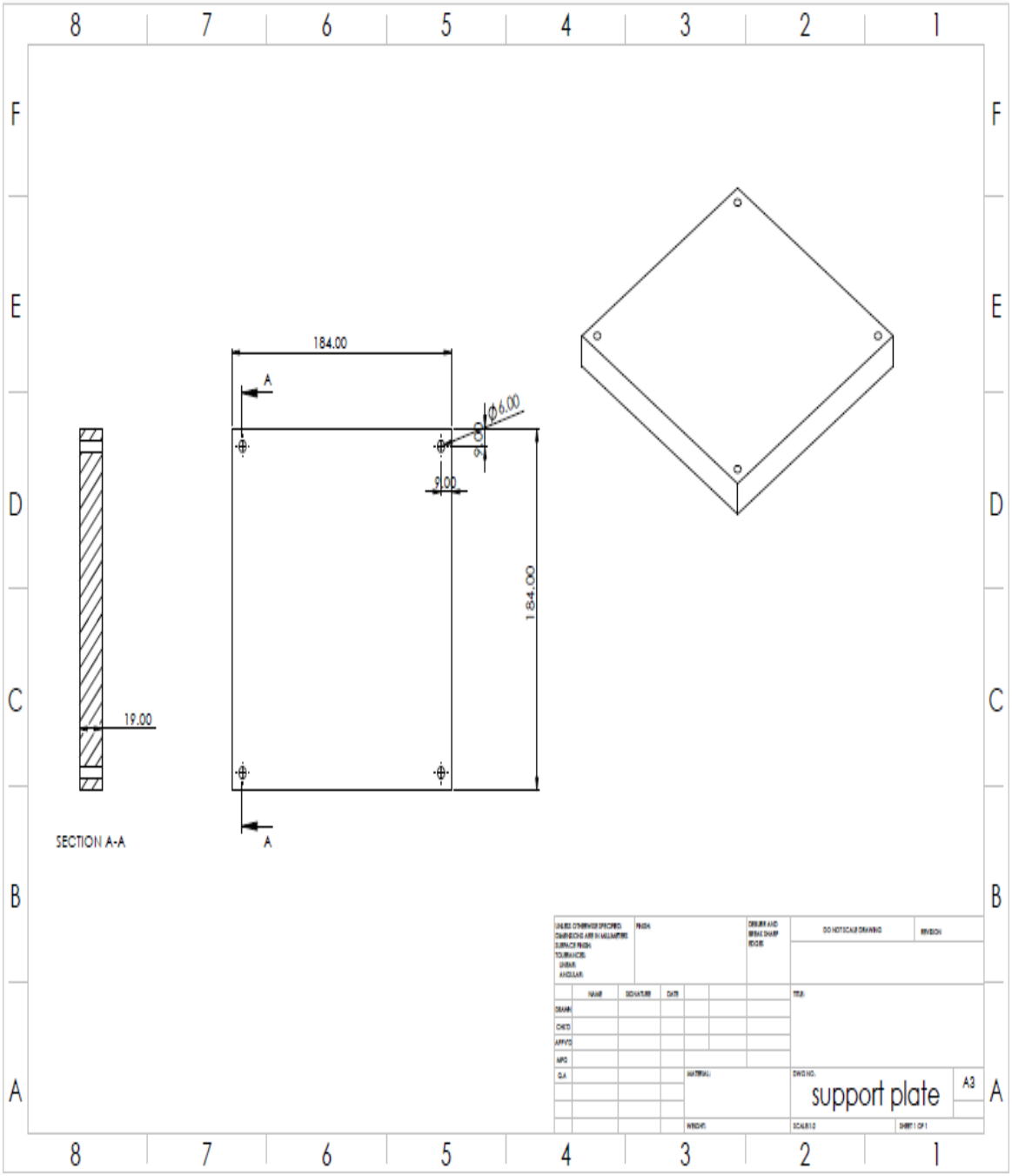

Figure 175: The design of the support plate.

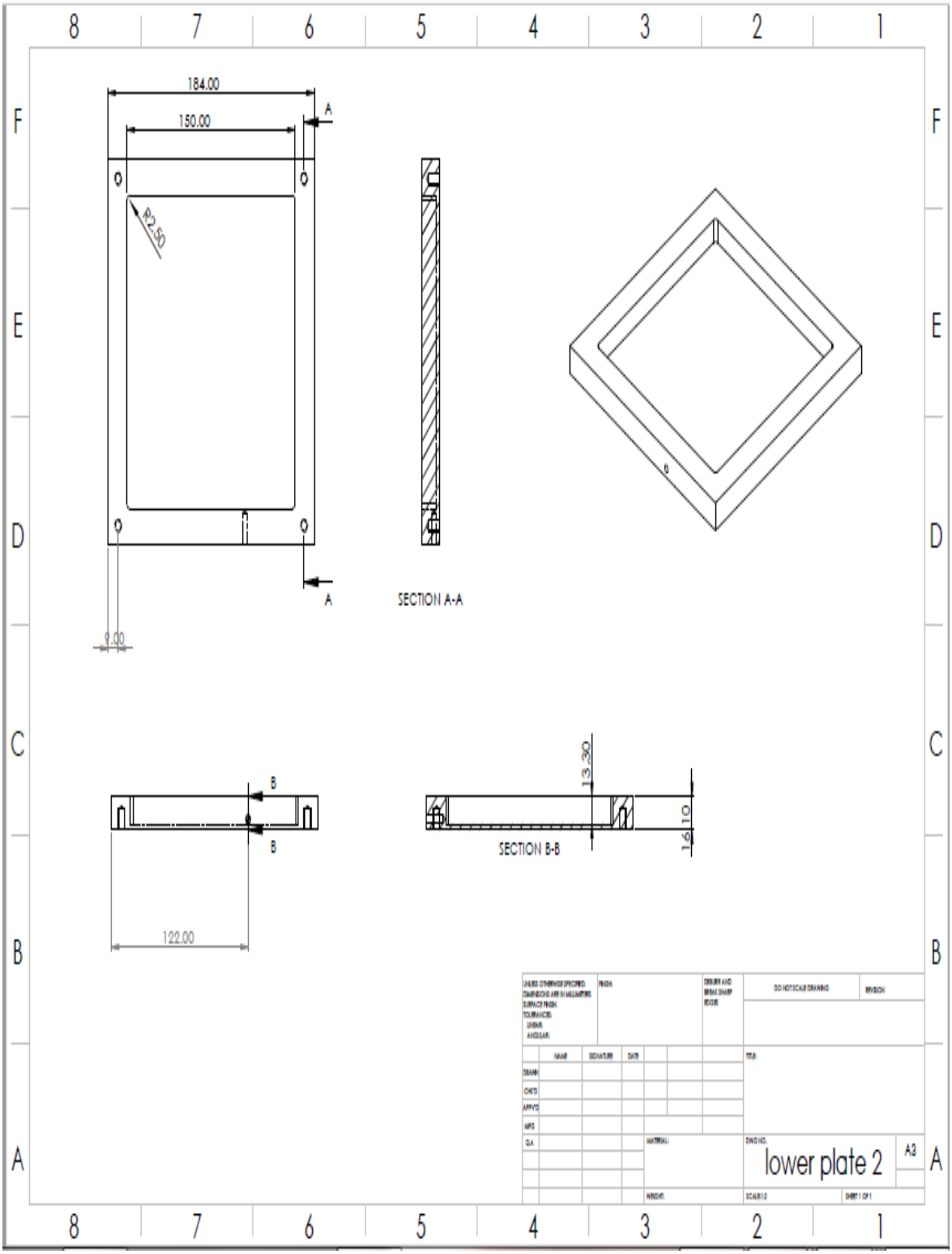

Figure 176: The design of the upper plate of the lower part.

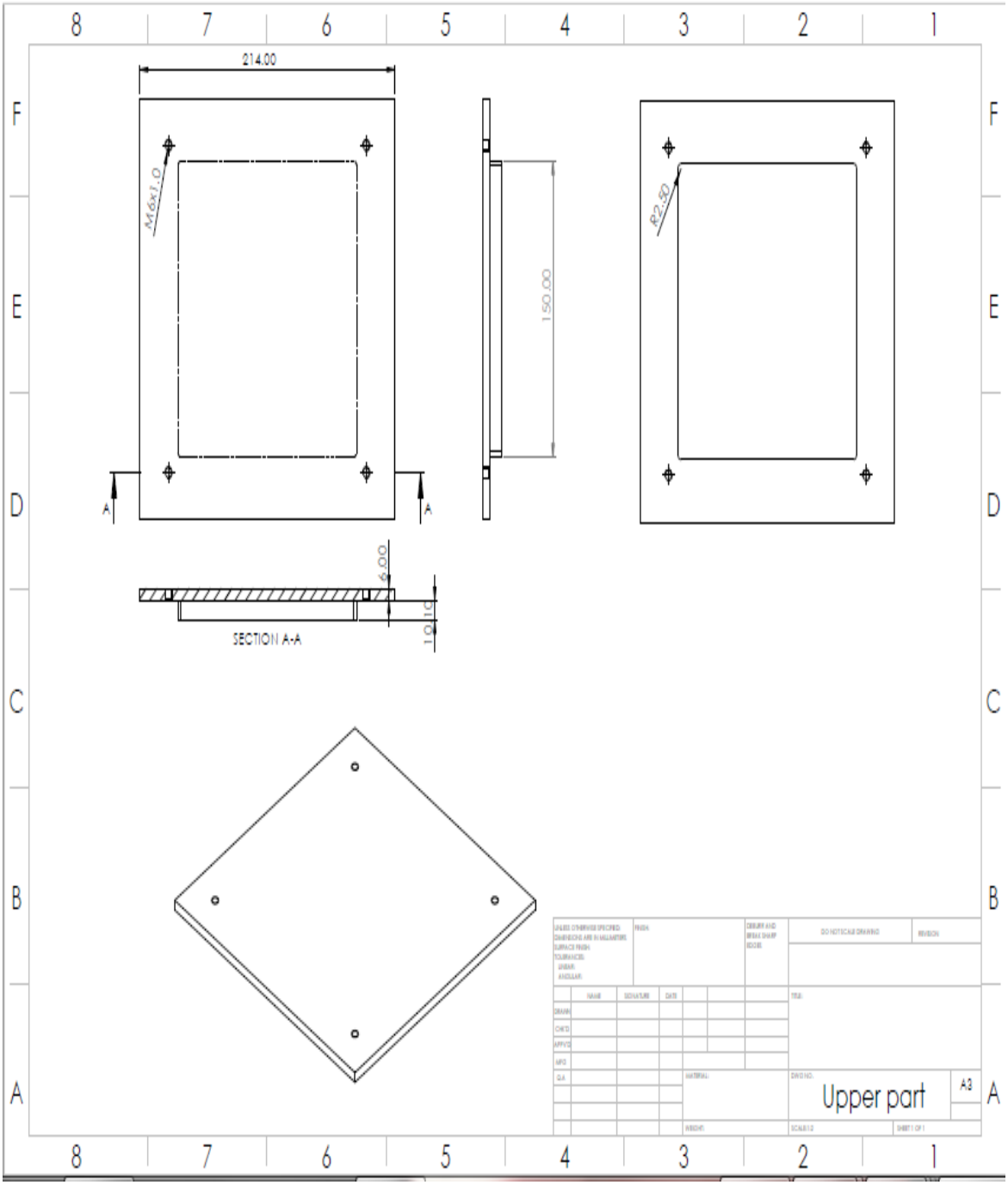

Figure 177: The design of the upper plate of the upper part.

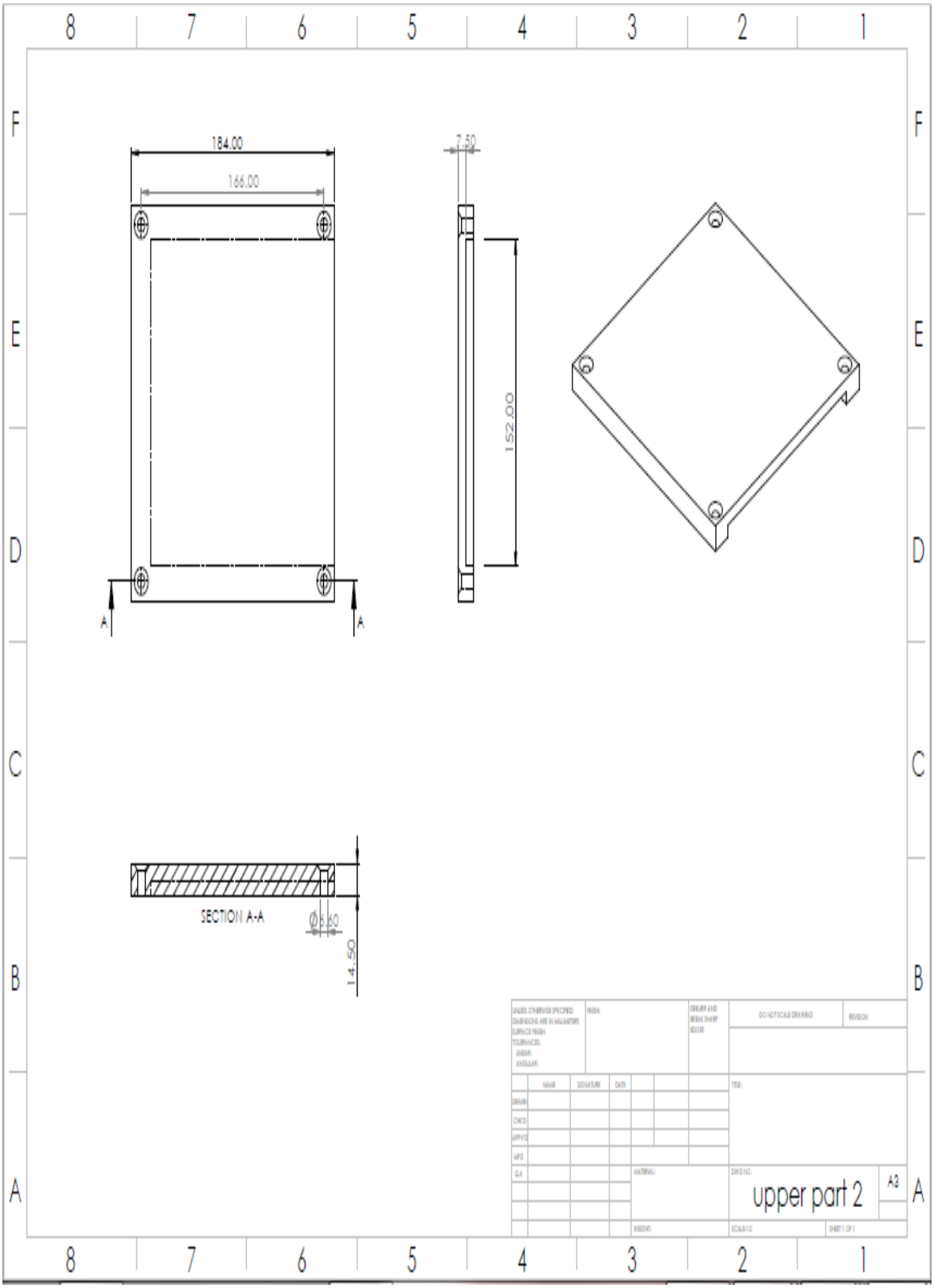

Figure 178: The design of the lower plate of the upper part.

Appendix D : The Experimental Procedures Of Each Of The Four Steps Of The AD Process Stage.

Step1: Starch And Fibres Separation Step

This step was carried out mainly for isolating starch and fibres from the biomass and make them ready for use, The following procedure was applied to each biomass separately:

1. Before peeling the material and separate the other residues (if any), the biomass was washed with clean water to remove any impurities like stones, metals, and holdfast. Following that, the peels were removed by stainless steel peelers.
2. In case of mango, the seed coats were opened to extract the seeds. The residues were then classified and separated into three groups: (a) peels and seed skins, (b) seeds and, (c) seed coats. The seeds were cut into quarters, blended with water and beaten in the Hollander beater to separate the starch.
3. Group (a) was the feedstock of the mango in AD process. After pre-treating group (b) the seeds, the leftover after separation of starch were added to group (a). While, the third group was stored in a container until it was processed to obtain mango seed coat fibres.
4. In case of avocado, the seeds were cut off into quarters and blended with water in the beater to extract the starch. The leftover after separation of starch, seed skins and the peels were mixed with each other and used as a feedstock for the avocado in the AD.
5. After blending, beating and filtering the processed seeds which contained starch in steps 2 and 4, these starch mixtures were left until the starch settled in the bottom of the container.
6. The powder which was tended to lighter colour settled down at the bottom of the container, while the remaining water and powder stayed on the top of it.
7. In order to prepare the biomass of each of the three fruits and vegetables and to isolate starch from potato peels, the peels were cut into small pieces.
8. Thereafter, the biomass was placed in the Hollander Beater and mixed with water at a ratio of (1:4) % w/w (in cases of mango and avocado, the biomass was mixed with the processed water of the starch separation). Note: the starch weight after separating it from the potato peel was considered in the measurements of the MS, TS, and VS %. The weights of the leftover cakes

which remained after separation of starch from mango and avocado seeds were also taken into account before blending it with the peels and water.

9. The gap between the drum and the bottom of the beater was adjusted by the crank handle to the minimum machine's gap (76 μm) and the beater was switched on.
10. Using a stop-watch, the biomass was then pre-treated for 5 mins.
11. During beating process, the top of the beater was covered. Weights were placed on the cover to avoid sprinkling of the biomass from the beater due to the vibration of the machine when it is running.
12. The drainage slot in the drum was opened after pre-treating the biomass to allow the mixture to discharge from the drum into a large plastic container. In case of potato peels, a strainer was placed at the drainage slot to isolate the starch.
13. The potato mixture which contained the starch was left for an hour in a container. The powder which was tended to lighter colour settled down at the bottom of the container, while the leftovers that remained in the strainer, were placed in a large container and used as a feedstock in the AD process.
14. Directly thereafter, a pre-determined quantities of the whole mango residues (peels, seeds, seed skins and seed coats) and avocado residues (peels and seeds) were processed separately in the beater. These residues were used as the controls (pre-treated biomass before the separation of starch from mango and avocado residues and coats from mango) in the AD processes of the mango residues and avocado residues. The same procedure described in the previous steps were followed with the controls but without separating the starch and coats or classifying the residues into groups.
15. The remaining water in step 13 was decanted it off into the large container which contained the leftovers.
16. The powder obtained after decanted off the water was soaked in 1% w/v sodium meta-bisulphite solution for 24 hrs and then washed thoroughly (adapted from [315]).
17. After washing and decanting off the water mixture which obtained from the previous step, the starch spread on a tray and air-dried until it was completely dry.

18. The darker colour powder on the surface of the starch powder was removed by a Laboratory scraper.
19. The starch powder was sieved using 1 mm grid sieve to obtain a fine starch powder.
20. The previous step was repeated if required.
21. The fine starch powder was then stored in an airtight container.
22. In order to prepare the fibres required, the seed coats in step 3 were sliced into small pieces and placed in a 5% NaOH solution at 90 ^{0}C for 2 hrs (adapted from [104]).
23. After thoroughly washing the resulted coats with water, they were dipped and stirred in a 1% (w/v) sodium meta-bisulphite solution for 24 hrs.
24. Following the chemical pre-treatment of the coats, they were washed thoroughly with clean water to ensure that the surface of the coats were free of $Na_2S_2O_5$ and any impurities and to prepare them for the mechanical pre-treatment.
25. The coats were then mechanically pre-treated by the Hollander Beater for 5 minutes.
26. In order to separate the large agglomerates of the pre-treated coats into small agglomerates, they were segmented into portions, blended in the electric blender one by one for 2 mins each at low and high speeds. To avoid heating of the blades, the blender was switched off for one minute after the first minute of blending to allow the blades to cool down. To measure the energy consumption of the fibre preparation process, the blender was connected to the electricity through an energy consumption meter.
27. Large fibres negatively affect the structure of the bio-plastic sheet, therefore, the blended coats portions were blended again one by one for 1 min at low and high speeds.
28. The previous step was repeated if required
29. The fibres were then spread on a tray and left until they were completely dry.
30. In order to ensure they were ready for use, the fibres were blended in a coffee mill, cut off into shorter length and stored in an airtight container.

Step2: Measurement Of The MS, TS And VS Of Each Biomass

The experimental procedure described below was followed to measure the moisture content MS, the dry matter of the samples TS and VS for each single biomass. The procedure was adapted from the NREL/MRI LAP (1994, 2008) standard methods [222, 288]. This procedure was applied on each biomass before and after the separation of starch and mango coats to find out the MS, TS and VS % of the control (before separation of the starch and fibres) and the biomass after the separations.

1. Seven empty beakers were weighed (one large for placing the biomass in, three medium for measuring MS, TS and VS % before separating the starch, and three small for measuring the MS, TS and VS % after separating the starch).
2. To measure the MS, TS and VS of the pre-treated residues of mango and avocado before and after the separation of the starch and coats, the pre-treated biomass in the large plastic containers were mixed carefully. Using the large beaker, approximately 2L of the biomass were taken randomly and directly after it was mixed. In the experiment of potato, after mixing the biomass the large beaker was also used for sampling from the beater directly (in case of the controls) and from the large container (in the case of the free-starch pre-treated potato wastes).
3. The biomass in the large beaker were mixed and 200 ml directly poured off in each of the six medium and small beakers.
4. The beakers were weighed using the electronic weighing scale. The weight of each of the empty six beakers was then subtracted from the weight of the beakers with the biomass to calculate the wet weight.
5. The six beakers placed in the oven for 24 hr at 105 ºC to measure the dry weight (TS).
6. After 24 hrs, the sample weights were measured, subtracted from the beakers weights and recorded. Another two readings of the samples dry weight were taken after one and two hours to ensure that the weight of the samples are constant, which indicates the sample had no moisture.
7. The average of the three readings were then calculated. This average TS weight is called “primary TS weight”.

8. The MS% of each of the six samples in the small and medium beakers were calculated by subtracting the wet weights from the dry weights, dividing the result by the wet weight, and multiplying by 100%.
9. The six dry samples were placed in Aluminium containers and combusted for 4 hours at 575 ± 25°C in the muffle furnace.
10. The weight of the ashes resulted from each sample was measured and subtracted from the Aluminium container weights.
11. The TS and VS were measured by applying the following equations,

$$\text{TS}(\%) = \frac{\text{dry weight}}{\text{wet weight}} \times 100 \qquad \text{Eqn. 38}$$

$$\text{VS}[\%\text{TS}] = \frac{\text{dry weight} - \text{ash weight}}{\text{dry weight}} \times 100 \qquad \text{Eqn. 39}$$

$$\text{VS}\% = \frac{\text{dry weight} - \text{ash weight}}{\text{wet weight}} \times 100 \qquad \text{Eqn. 40}$$

12. In order to unify the VS weight of all biomass and therefore, simplify the comparison between the optimal biogas produced from each biomass, the average TS weight resulted (the primary TS weight) was then used to find out the TS weight at each of the organic concentration level. The following equation was applied to calculate the weight of the dry matter (TS weight) at each level of the organic concentration factor) based on the primary TS weight in step 7.

$$\text{Average TS weight at each organic concentration level} = \frac{\text{The primary TS weight} * \text{the organic concentration level required}}{\text{Average VS weight at the primary TS weight}} \qquad \text{Eqn. 41}$$

13. The following equations were applied to adjust the VS by water dilution at the required levels.

$$C_1\,[\%] * V_I\,[\text{ml}] = C_2\,[\%] * V_2\,[\text{ml}] \qquad \text{Eqn. 42}$$

$$V_2\,[\text{ml}] = V_1\,[\text{ml}] + D\,[\text{ml}] \qquad \text{Eqn. 43}$$

Where, C_1 is the organic concentration was resulted, C_2 is the concentration required, V_1 the volume of the mixture at C_1, V_2 is the volume of the mixture at C_2 and D is the amount required to be added/ removed to adjust the VS at the predetermined concentration.

14. The same procedure was repeated after adjusting the organic concentration (VS%) mathematically to make sure the pre-determined VS% has been obtained.

Step3: Anaerobic Digestion Process

The following procedure was applied to each biomass.

1. Before distributing the flasks in the water paths, the pH level of each sample was measured. Thereafter, the flasks were distributed, closed and sealed carefully with, red clips, and a stopper to prevent leakage of gas.
2. All flasks were attached to the gas collection system with the glass bores taps.
3. Following that, each flask was connected to one Aluminium bag. The bags must be ensured that are tightly sealed to prevent any leakage of gas, prevent entry of air into the bags, and for safety purposes.
4. To control the movement of the gas, a three ways valve was placed between the reactor and bags.
5. To provide airtight environment and expel air from the system, nitrogen was pumped into all Aluminium bags and reactors.
6. In order to prevent the contamination of air, the growth of the anaerobes in the sludge and the presence of oxygen gas, electric vacuum pump was applied for extraction the nitrogen gas and the atmospheric air from all bags and reactors.
7. The last two steps were repeated three times for each reactor for ensuring the system is free of air.
8. All water baths were then filled with water up to the maximum level and their temperature set according to the design matrix.
9. Water levels in all water baths were checked on a daily basis. As water level was reduced, water was added up in the bath for maintaining the water at the maximum level.
10. Slow shaking of reactor on a daily basis was importantly required to enhance the biological reaction within the reactors in the digestion period.
11. The biogas produced from each reactor collected twice over the period of the AD process.

Step4: Measurement Of The Biogas Produced

The following steps were followed in the collection and measurement of the volume and the concentrations of biogases produced.

1. The reactor was connected to the gas measurement apparatus (volumetric flask, round bottom flask, and electric pump) through three ways valves.
2. The conical flask valve, round bottom flask valve, and the bag valve setting were isolated. All air contaminations inside the apparatus should be removed by circulating nitrogen gas for approximately 2 minutes. The nitrogen inside the volumetric flask and in the round bottom flask were removed by vacuum pump.
3. The inverted inner cylinder was then fully inserted downward in the outer cylinder. The gas in the bag was released through opening the valve.
4. The reading of the water level on the scale in the inverted cylinder was recorded (initial reading).
5. A vacuum was created by lifting up the inverted inner cylinder out of the outer cylinder and holding it. The reading at which the water crossed the inverted cylinder was recorded (final reading). Note: for this step it is important to ensure that the Aluminium bag is completely free of any gas.
6. The aluminium bag valve was then closed, and the inverted inner cylinder was fully inserted downward in the outer cylinder to force the measured gas into an external aluminium bag. The gases measured in step 4 and 5 were stored in the external bag.
7. The biogas detector was attached to the external Aluminium bag to measure the content of the biogas produced of the each gas (CH_4, CO_2, O_2, etc.).
8. The volume of biogas was calculated by subtracting the initial reading in step 4 from the final reading in step 5.
9. For ensuring the stability of the digester and the quality of the digestate, the pH of the digestate produced from each sample was measured by pH meter.
10. This procedure was replicated with each reactor, to measure the volume of the biogas and its content.

Appendix E : The Experimental Procedures Of The Manufacturing Of The Bio-Plastic Sheets.

The following steps are the procedure followed for producing the bio-plastic sheet:

1. The weight of the starch based on the quantities and types shown in the design matrix for each sheet was placed in a small plastic container.
2. Glycerine in a percentage of 30% of the initial weight of the starch was blended with the starch in the container using scale syringe.
3. The mixture of the starch and the glycerine were manually stirred until it is ensured the full wetting of starch in glycerol is occurred.
4. Distilled water was added to the mixture in a percentage of 12% of the initial weight of the starch using scale syringe and stirred manually for separating the large agglomerations.
5. The mixture then blended with the pre-specified weight of fibres. The pre-specified amount of fibre was added gradually to the mixture.
6. In order to ensure a good incorporation occurred between the fibres and the starch/glycerol/water, the mixture was stirred until a good incorporation was seemed to be achieved.
7. Two non-stick paper sheets were then cut off according to the sizes of the upper and lower plates of the mould. After placing the paper sheet in the lower plate, the mixture was poured into the lower plate with careful distributions of the mixture.
8. The other non-stick paper sheet was placed above the mixture. Following that, the upper plate was carefully covered the lower plate and pressed manually.
9. After adjusting the temperature of the mould to 140 °C ± 2 °C, the mould was placed on the press base, compressed hydraulically and heated for one hour.
10. The mould was then de-pressed, placed on an Aluminium tray for 25 ± 5 minutes to allow the sheet produced to cool down. The sheet was then de-moulded, placed on the tray and a 500g weight was placed over it to prevent it from bending.

11. On the next day, the sheet produced was cut according to the Standard Test methods used for each of the test using the CO_2 laser cutting machine.

Appendix F : The Drawing Of The Specimen Dimensions For Each Of The Four tests, A) Tensile Test, B) Flexural Stiffness Test, C) Impact Strength Test And D) Density Test.

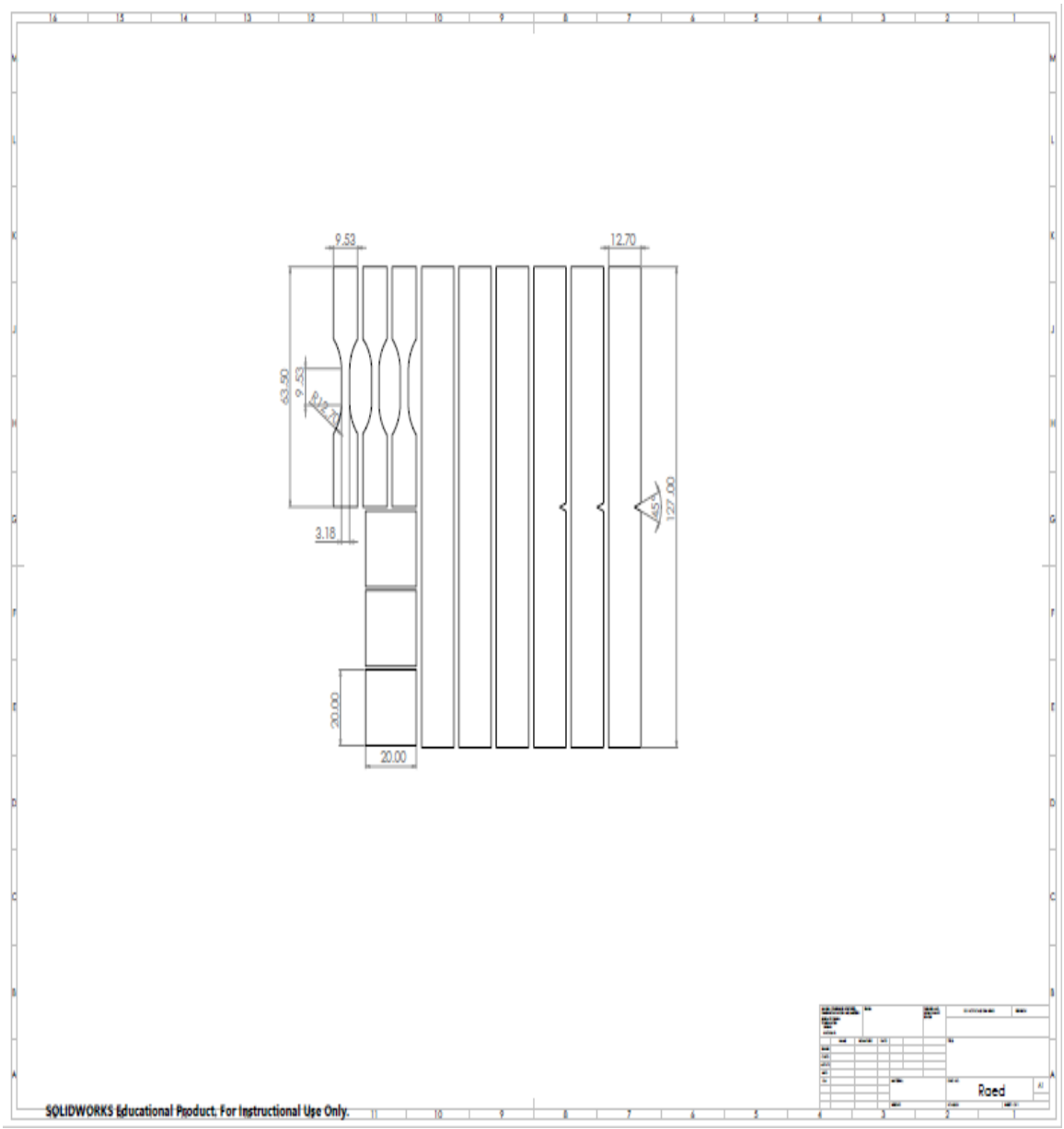

Figure 179: The drawing of the specimen dimensions for each of the four tests.

Appendix G : The TS%, MS% And The Adjusted Weights Of The VS And TS At Each VS Level Of The Pre-treated Biomass Before And After The Separation Of Starch And fibres.

Table 59: The TS, MS, and VS% before separation of the starch from the potato residue.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	TS weight, g	TS %	MS %	Ash weight, g	VS weight, g	VS % (TS)	VS %
1	107.80	314.80	207.00	6.79	3.28	96.72	0.57	6.22	91.61	3.00
2	106.60	332.70	226.10	8.26	3.65	96.35	0.69	7.57	91.65	3.35
3	105.90	328.80	222.90	7.20	3.23	96.77	0.64	6.56	91.11	2.94
Average				7.42 ±0.61	3.39 ±0.18	96.61 ±0.18		6.78 ±0.57	91.45 ±0.24	3.10 ±0.18
Adjusted weight				7.11 4.43 1.75				6.50 4.05 1.6		

Table 60: The TS%, MS, and VS% after separation of the starch from the potato residue.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	TS weigh, g	TS %	MS %	Ash weight , g	VS weight, g	VS % (TS)	VS %
1	106.00	319.60	213.60	6.51	3.05	96.95	0.42	6.09	93.55	2.85
2	106.80	326.90	220.10	7.33	3.33	96.67	0.44	6.89	94.00	3.13
3	109.30	328.80	219.50	7.25	3.30	96.70	0.43	6.82	94.07	3.11
Average				7.03 ±0.36	3.23 ±0.12	96.77 ±0.12		6.60 ±0.36	93.87 ±0.23	3.03 ±0.12
Adjusted weight				6.92 4.31 1.70				6.50 4.05 1.6		

Table 61: The TS%, MS, and VS% before separation of starch and fibre from the mango residue.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	TS weight, g	TS%	MS%	Ash weight, g	VS weight, g	VS% (TS)	VS %
1	107.8	324.8	217	6.92	3.19	96.81	0.32	6.60	95.38	3.04
2	108.2	320.5	212.3	6.55	3.09	96.91	0.32	6.23	95.11	2.93
3	108.7	321.6	212.9	6.79	3.19	96.81	0.35	6.44	94.85	3.02
Average				6.75 ± 0.15	3.15 ±0.04	96.85 ±0.04		6.42 ±0.15	95.11 ±0.21	3.00 ±0.04

Adjusted weight				6.83 4.25 1.68				6.50 4.05 1.6		

Table 62: The TS, MS, and VS% of the samples after separation of the starch and fibres from the biomass.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	Average TS weight, g	TS%	MS%	Ash weight, g	VS weight, g	VS% (TS)	VS %
1	105.1	326.9	221.8	7.65	3.45	96.55	0.30	7.35	96.08	3.31
2	106	321.1	215.1	7.03	3.27	96.73	0.28	6.75	96.02	3.14
3	105.4	319.6	214.2	7.13	3.33	96.67	0.26	6.87	96.35	3.21
Average				7.27 ±0.27	3.35 ±0.07	96.65 ±0.07		6.99 ±0.25	96.15 ±0.14	3.22 ±0.07
Adjusted weight				6.76 4.21 1.66				6.50 4.05 1.6		

Table 63: The TS%, MS, and VS% of the samples before separation of the starch from the avocado residue.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	TS weight, g	TS %	MS %	Ash weight, g	VS weight, g	VS % (TS)	VS %
1	108.50	313.01	204.51	6.61	3.23	96.77	0.37	6.24	94.40	3.05
2	109.30	318.48	209.18	6.99	3.34	96.66	0.41	6.58	94.13	3.15
3	107.50	314.92	207.42	6.81	3.28	96.72	0.39	6.42	94.27	3.10
Average				6.80 ±0.15	3.29 ±0.04	96.71 ±0.04	0.39 ±0.01	6.41 ±0.13	94.27 ±0.11	3.10 ±0.04
Adjusted weight				6.90 4.30 1.70				6.50 4.05 1.6		

Table 64: The TS, MS, and VS% after separation of the starch from the avocado residue.

#	Beaker weight, g	Wet weight with beaker, g	Wet weight, g	TS weigh, g	TS %	MS %	Ash weigh, g	VS weigh, g	VS % (TS)	VS %
1	106.00	321.40	215.40	7.11	3.30	96.70	0.36	6.75	94.94	3.13
2	106.60	324.70	218.10	6.49	2.98	97.02	0.38	6.11	94.14	2.80
3	106.20	324.00	217.80	6.90	3.17	96.83	0.36	6.54	94.78	3.00

Average	6.83 ±0.25	3.15 ±0.13	96.85 ±0.13	0.37 ±0.01	6.47 ±0.2	94.62 ±0.34	2.98 ±0.1
Adjusted weight	6.87 4.28 1.69				6.50 4.05 1.6		

Appendix H : The Results Of All Responses Of Each Biomass Including The pH Levels After Digestion Of Samples

Table 65: The pH levels and the results of all responses of the biogas produced from each sample.

Std	Run	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4,%	CO_2,%	CH_4 content cc/g-VS
1	4	7.9	835.7	522.3	52.1	33.2	272
2	16	8.0	1000.2	625.1	49	36.5	306.5
3	3	6.7	1592.7	245	19.1	58.8	46.8
4	7	6.8	1803.8	277.5	18.5	59.2	51.4
5	14	6.8	1543.6	381.1	22.1	40	84.2
6	6	6.5	1619.2	399.8	20.1	46.1	80.4
7	5	8.1	2676.9	661	61.8	22.4	408.3
8	2	8.2	3204.1	791.1	59.8	29.6	473.1
9	9	7.9	1087.9	679.9	48.1	34.6	327.1
10	1	6.6	2261.3	347.9	10.2	68.1	35.5
11	10	8.1	2236.7	1397.9	69.2	22.1	967.4
12	15	8.0	2953.6	454.4	57.3	27.7	260.4
13	11	8.1	2177.8	537.7	65.1	22.5	350.2
14	12	7.9	2171.1	536.1	64.7	20.5	346.8
15	17	8.0	2253.3	556.4	64.8	24	360.3
16	8	8.1	2037.8	503.2	64.7	20.6	325.5
17	13	7.9	2396.6	591.8	68.1	22.7	403

Table 66: The pH level and results of all responses of the biogas produced from each samples of mango residues.

Std	Run	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4,%	CO_2,%	CH_4 content cc/g-VS
1	12	7.7	1090.7	681.7	54.9	32.8	374.5
2	9	7.7	1193.4	745.9	59.6	25.6	444.3
3	13	6.9	1786.9	274.9	44.8	44.9	123.3
4	4	7.0	2468.7	379.8	51.3	38.2	195
5	8	7.0	1293.6	319.4	47.0	36.8	150.1
6	6	7.3	1726.5	426.3	56.9	32.4	242.4
7	15	7.8	2842.7	701.9	52.0	29.5	364.6
8	10	8.0	3379.7	834.5	61.9	28.8	516.5
9	1	7.4	704.2	440.1	53.8	30.3	236.9
10	2	6.5	861.9	132.6	20.4	61.0	27.0
11	3	7.9	1574.4	984.0	42.8	40.8	421.2
12	7	7.9	3724.5	573.0	54.6	30.1	313.0
13	5	7.8	1916.1	473.1	68.9	25.8	326.0
14	14	7.7	1988.1	490.9	65.9	25.9	323.5
15	11	7.7	2013.3	497.1	65.4	26.1	325.1
16	17	7.6	2038.8	503.4	65.8	26.2	331.1
17	16	7.6	1881.6	464.6	65.5	22.8	304.2

Table 67: The pH levels and the results of all responses of the biogas produced from avocado residues.

Std	Run	pH level	Total Biogas, cc	Biogas, cc/g-VS	CH_4, %	CO_2, %	CH_4 content, cc/g-VS
1	16	7.7	1484.0	927.5	52.1	30.4	483.5
2	10	7.7	1672.1	1045.1	51.3	30.3	535.8
3	14	7.6	3542.9	545.1	34.8	48.8	189.7
4	9	7.7	3832.1	589.5	62.2	28.3	366.5
5	6	7.6	1617.3	399.3	42.6	36.8	170.1
6	13	7.5	1913.3	472.4	45.1	35.9	213.1
7	7	7.8	3404.1	840.5	40.3	42.6	338.7
8	3	7.8	3571.6	881.9	56.6	25.3	499.1
9	12	7.6	1107.1	691.9	61.9	22.2	428.3
10	15	7.1	2193.2	337.4	46.1	36.8	155.4
11	5	8.0	1879.5	1174.7	56.9	27.2	668.0
12	8	8.0	4891.9	752.6	61.1	29.4	459.6
13	11	7.8	2585.9	638.5	62.0	21.2	395.7
14	2	7.8	2547.5	629.0	62.4	21.3	392.7
15	4	7.8	2594.4	640.6	61.7	21.7	395.0
16	17	7.8	2555.7	631.0	59.2	20.6	373.4
17	1	7.7	2637.9	651.3	58.8	22.3	382.8

Appendix I :The Chemistry Laboratory Certificate On The Content Of The Resulting Digestate To The Three Main Nutrients.

ENVIRONMENTAL CHEMISTRY TEST CERTIFICATE

Report Status: **Final Report**
Date of Issue: **30-Mar-2018**
Report Number: **473250**
Project: **1-180316-06733**
Page 1 of 1

Attention:
Client: **DCU**
Address: Glasnevin,
Whitehall,
Dublin 9

Order Number:

Disclaimer
Results in this report relate only to the items tested.
Reports may not be reproduced in full without the approval of **Advanced** Laboratory **Testing**.
Results reported as cfu/cm2 are calculated based on information supplied by the relevant customer regarding the specific area swabbed.
* beside the method or lack of INAB symbol signifies that **Advanced** Laboratory **Testing** are not INAB accredited for this method.
Samples are retained post analysis for a period of 10 days. Samples are stored frozen by default except in the case of M&S and customer

Project Comment: Results reported are based on sample as received.

ALT ID: 1189818 **Date Received:** 16/03/2018 **Date Tested:** 22/03/2018
INAB P9 Classification: Others: Digestate
Client ID: Liquid digestate generated after anaerobic digestion of a mixture of mango peels and sludge at temperature 35 C, organic weight 6.58g and sludge 200ml

Test	Result	Unit(s)	Method	Technique
Total Phosphorous	762	mg/kg	ECTM008	HACH Method 8190
Potassium	1251	mg/kg	ECTM023	In-House
Dry Matter	3.2	g/100g	ECTM024	In-House
Total Nitrogen	3951	mg/kg	ECTM025	In-House

Figure 180: The certificate of the chemistry Lab for the results of the tests of the digestate.